The survival and transfer of potentially pathogenic bacteria from environmental sites and surfaces.

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THE SURVIVAL AND TRANSFER OF POTENTIALLY PATHOGENIC BACTERIA FROM ENVIRONMENTAL SITES AND SURFACES

CONTENTS

PUBLICATIONS IN SUPPORT OF THIS THESIS

1. THE SURVIVAL AND TRANSFER OF MICROBIAL CONTAMINATION VIA CLOTHS, HANDS AND UTENSILS. Elizabeth Scott & Sally F Bloomfield. Journal of Applied Bacteriology, 1990, 68, 271-278

2. INVESTIGATIONS OF THE EFFECTIVENESS OF DETERGENT WASHING, DRYING AND CHEMICAL DISINFECTION ON CONTAMINATION OF CLEANING CLOTHS. Elizabeth Scott & Sally F Bloomfield. Journal of Applied Bacteriology. 1990, 68, 279-283

3. THE SURVIVAL OF MICROBIAL CONTAMINATION ON WORKSURFACES AND CLOTHS AND ITS TRANSFER VIA CLOTHS, HANDS AND UTENSILS. Elizabeth Scott & Sally F Bloomfield. Environmental Health 1990, Feb. 34-37

4. A BACTERIOLOGICAL INVESTIGATION OF THE EFFECTIVENESS OF CLEANING AND DISINFECTION PROCEDURES FOR TOILET HYGIENE. Elizabeth Scott & Sally F Bloomfield. Journal of Applied Bacteriology 1985, 59, 291-297

ABSTRACT

The introduction to the thesis describes the historical development of current attitudes towards control of infection with particular reference to food-borne disease and hospital acquired infection. The role of the inanimate environment in the transfer of infection is defined and the risk to the community is outlined with particular reference to food and pharmaceutical manufacture and preparation and the clinical situation.

Current approaches to controlling environmental contamination and preventing the transmission of infection by the use of barriers and biocides are described.

The thesis describes experimental studies undertaken on three environmental sites, namely work surfaces, cloths and toilets and wastetraps. The aim of these studies is as follows:

1. To determine the extent to which bacterial contamination can survive at each type of site
2. To determine the potential for transfer of bacterial contamination from each site, especially via hands, cloths and other inanimate objects, to potentially more hazardous sites and surfaces
3. To determine the effectiveness of certain disinfection procedures in breaking the chain of transmission of bacterial contamination from environmental sites

The method of study includes both laboratory experiments using model work surfaces and also procedures carried out in the field. A chapter on methodology includes a discussion of the relative merits of the microbiological

sampling techniques employed. Experimental results are compared with those of other workers.

The results of these investigations are discussed and their implications in relation to current methods in control of infection and contamination in hospital, food processing and pharmaceutical manufacturing environments are considered.

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LIST OF FIGURES AND TABLES

(abbreviated)

CHAPTER 1

INTRODUCTION

1.1 Historical Introduction

At the time of writing this thesis, we are going through a period of intense national concern about the increasing number of food poisoning cases in England and Wales. During the past year the Government has set up an independent committee to advise on problems of food poisoning, especially those caused by salmonella, listeria and campylobacter.

Food poisoning is not a new disease and knowledge of some of the causes of food poisoning dates back many centuries. Documented history of disinfection and hospital infection control indicates that our understanding of the infection risk presented not only by contaminated food and water but also by our inanimate environment also dates from early times.

In studying the subjects of food poisoning and hospital acquired infection there is much common ground and a review of the historical development of infection and infection control methods reveals considerable overlap.

1.1.1 History of Food Poisoning

Noxious substances in food give rise to an illness called food poisoning or gastroenteritis, which is usually characterized by vomiting and/or diarrhoea together with various abdominal disturbances. As stated earlier, the need to control food poisoning has been recognized through the ages as described in the laws of the Israelites. About 2000 BC, as recorded in the Book of Leviticus, Moses laid down rules stating which types of meat were clean and safe to eat (Leviticus Chap. 11) and that only freshly slaughtered meat should be consumed (Leviticus 7.24). The book of Leviticus also

details rules about washing hands, clothing and vessels before and after sacrificing animals and before eating (Leviticus 6.27 and 28, 8.6). Many of these rules must have been based on a practical knowledge of personal hygiene.

By contrast, specific knowledge of non-chemical, that is bacterial food poisoning, dates only as far as the latter part of the nineteenth century (Hobbs & Roberts 1987) and followed as a consequence of the development of the science of microbiology. Early pioneers in this field included Louis Pasteur and Robert Koch. In 1859, Pasteur demonstrated the role of bacteria in the fermentation process and developed laboratory methods for growing bacteria for study. Of particular importance was his work which showed that earlier theories of spontaneous generation were false and that if food was sterilized by heat, living bacteria would not reappear unless introduced from an outside source. At about the same period, Koch proved that anthrax, tuberculosis and cholera were caused by bacteria and other microbiologists around the world identified the causative organisms of many other diseases.

Important dates in relation to the purity of public water supplies include the publication of a Report on the Sanitary Conditions of the Labouring Population of Great Britain in 1842 by Edwin Chadwick and the recognitition by John Snow in 1854 that drinking water was involved in the spread of cholera. In 1856, William Budd recognized that typhoid fever was spread by contaminated milk or by water polluted by the excreta of an infected person.

Perhaps the most important contribution to public health was the introduction of chlorination of drinking water in Britain which was initiated by Alexander Houston in 1905.

The first description of food poisoning bacteria was given by Gaertner in Germany in 1888 when he isolated bacteria from the organs of a man who had died from food poisoning. Similar bacteria were found in the meat served to the victim and also throughout the carcass from which the meat had originated. The bacteria were later named Salmonella. At about the same time the ptomaine theory of food poisoning was disproved when volunteers consumed ptomaines or alkaloids, extracted from putrid food without ill effects. From then on, food poisoning became associated with specific bacterial contamination.

In 1896, E. van Ermengem in Belgium described Clostridium botulinum, the organism responsible for botulism. The toxin from this organism, formed in certain imperfectly preserved foods, affects the central nervous system and is often fatal. Botulism is rarely reported in the UK nowadays, because the home preservation of meats, fish and vegetables by canning and bottling is discouraged.

In the years 1909 to 1923 many of the bacteria now known to be responsible for a large proportion of food poisoning incidents were grouped together under the generic name Salmonella in honour of Dr. E. Salmon, who isolated the first member, the hog cholera bacillus, in 1885.

From 1914 onwards the staphylococci became associated with a toxic form of reaction which gives rise to the

rapid onset of vomiting. Certain strains of staphylococci can produce a toxin in food without visible or flavour effect and this often occurs in cooked foods such as cold meats and dairy products such as cream and custard. By 1953, Clostridium perfringens was recognized as an agent of food poisoning with diarrhoea as the predominant symptom.

Other organisms more recently recognized as agents of food poisoning include Bacillus cereus, Escherichia coli, Campylobacter jejuni and Listeria monocytogenes.

In 1950, comprehensive records for food poisoning outbreaks and incidents were initiated when reports to the Public Health Laboratory Service (PHLS) and the Ministry of Health were combined. In 1968, notification of outbreaks of food-borne disease was made a statutory requirement. For England and Wales, the food poisoning data based upon laboratory investigation and report is published by the PHLS Communicable Disease Surveillance Centre (CDSC) and in Scotland by Communicable Disease Scotland (CDS). The statutory notifications of food poisoning sent to the Office of Population Censuses and Surveys (OPCS) are based on clinical diagnosis or suspicion of food poisoning and clinical specimens for laboratory investigation may not have been taken.

Communicable Disease Reports (CDR) are published weekly by CDSC from laboratory investigated cases and reveal information on causative organisms, place and cause of outbreak and suspected vehicle of infection etc. The number of cases of food poisoning is published under the heading "All cases" (a 'case' is a person with symptoms who is excreting food poisoning organisms) and this is

further divided into the following headings:

"General Outbreaks" : two or more cases in different families

"Family Outbreaks" : two or more cases confined to the same household

"Sporadic Cases" : Single cases which are isolated occurrences having no connection with other cases

Over the last 10 years, the food poisoning statistics indicate annual increases in the number of cases. This has been due largely to rises in numbers of reports of salmonella and campylobacter infection as shown in Fig.1. Salmonella reports rose from 10,761 in 1980 to 25,831 in 1989 (PHLS unpublished data). Most of this increase was due to increased reporting of Salmonella enteritidis phage type 4, after 1985. Reports of campylobacter infections rose from 9,453 in 1980 to 32,890 in 1989 (PHLS unpublished data).

It has become widely accepted that the numbers of food poisonings are generally underestimated and that the real figures could be anything from 5 to 100 times the reported total.

It is likely that a combination of factors is responsible for the upward trend of food poisoning statistics. Sheard (1986) listed the following likely factors:

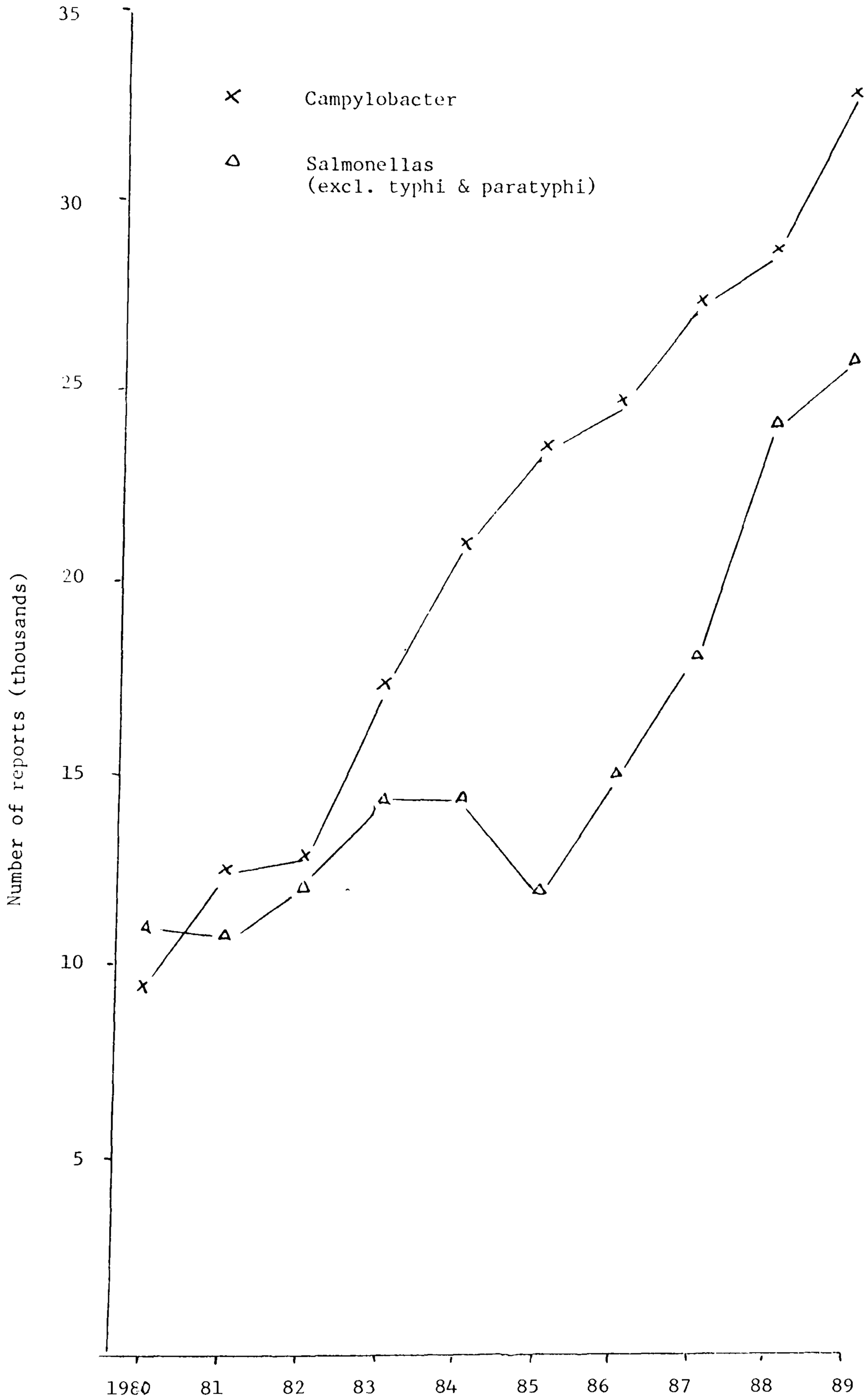
a) intensive farming of meat and poultry

b) larger quantities of meat and poultry passing through slaughterhouses and processing units

c) lack of training amongst catering staff

d) lack of adequate temperature control of foodstuffs

Fig 1. Reported Salmonella and Campylobacter infection 1980-1989



Reference PHLS unpublished

Points c) and d) above refer to a lack of food hygiene training and practice and this is supported by a survey of the causes of food poisoning during 1970-1979 in which Roberts (1982) listed the following ten most common contributory factors for food poisoning:

1) Food prepared too far in advance
2) Food stored at room temperature
3) Cooling food too slowly prior to refrigeration
4) Not reheating food to a high enough temperature to destroy food poisoning bacteria
5) The use of cooked food contaminated with food poisoning bacteria
6) Undercooked meat and meat products
7) Insufficiently thawed frozen meat and poultry
8) Cross-contamination from raw to cooked foods
9) Hot food stored below 63°C
10) Infected food handlers

More recently other contributory factors including the problems of contaminated animal feedstuff, the contamination of shell eggs with S. enteritidis PT4 and the development of new technologies such as the cook-chill process for the preparation of the so-called 'convenience foods' have been identified.

1.1.2 History of Hospital Infection Control

The early development of infection control in the clinical environment is generally associated with Ignaz Semmelweis and his successful attempts to reduce the incidence of puerperal or childbed fever in the Vienna General Hospital in the mid nineteenth century.

The conditions under which Semmelweis worked were very poor compared with those of a modern hospital (Reid 1974). In 1844, almost 10% of women admitted to the maternity ward at the Vienna General died of puerperal fever. As in all hospitals at that time, wards were crowded and conditions were extremely primitive. Toilet closets with open sewers or buckets led directly from the wards and in many cases, the autopsy rooms were directly adjoining to the wards. There was no tradition of cleanliness either with respect to patients, practitioners or surroundings. Surgeons did not necessarily clean or change aprons between operations and wards were washed monthly or yearly according to the whim of the nurses. In the maternity unit of the hospital there were two clinics, one was a teaching clinic with medical students and the second was run by midwives in the same way as the teaching clinic but without medical students. Semmelweis observed that over a period of six years the death rate from childbed fever was 10% in the teaching clinic compared with 3% in the midwives clinic. Semmelweis postulated that the cause of the infections was carried on the hands of the students and teachers from autopsies to examination of women in labour. He instituted a strict handwashing procedure using chlorinated lime and as a result mortality in the teaching clinic was reduced from 11% in 1846 to 3% in 1847. Following further episodes of infection, handwashing between patients as well as between autopsy room and ward was introduced together with the isolation of women with badly infected wounds etc. Eventually mortality from puerperal fever was reduced to 1.3%.

Records from the middle of the nineteenth century suggest that the primary casue of death following surgery was infection. There was little or no understanding of how the so-called 'hospital diseases' such as erysipelas, pyaemia, septicaemia and gangrene were spread apart from the knowledge that dirt and overcrowding increased the risk of disease.

In recognition of this, Florence Nightingale introduced an order of cleanliness to the poor hospital conditions of the British Army during the Crimean War of 1853-1856. As a result, the Crimean War hospital death rate was reduced from 42% to 2% (Hutchinson Encyclopaedia 1988). In 1865, Joseph Lister, a surgeon at Glasgow Infirmary, read of Pasteur's experiments proving that living matter or germs in the air were responsible for process of fermentation and putrefaction in food. From this, Lister postulated that if germs could be prevented from gaining access to the wound it might be possible to prevent the 'hospital diseases' which followed putrefaction. He reasoned that if wounds were covered with a dressing which did not exclude the air but killed off the particles suspended in it, this could provide a way of reducing death and disease. The method he devised (Lister 1867) was the application of carbolic dressing to wounds. These were the first antiseptic dressings and with them Lister initiated the era of antiseptic surgery.

Nearly one hundred years after Lister recognized the serious risks presented by microorganisms in the hospital environment, the Standing Medical Advisory Committee to the Ministry of Health recommended the appointment of Control of Infection Officers to hospitals (HMSO January 1959). Together with another recommendation for the

setting up of Control of Infection Committees within hospitals, this was one of many recommendations made as a result of serious cross-infection problems and in particular the need to control staphylococcal infections in hospitals. The Control of Infection Officer was identified as a key position in the co-ordination of regular preventative measures and the control of outbreaks. In April 1959, the first ever Infection Control Nurse was appointed at Torbay Hospital. In 1960, a second appointment was made at the Royal Devon and Exeter Hospital. Gradually, appointments were made throughout the country and in 1966 a study conference was held at Taunton, attended by fourteen infection control nurses. In 1970, the Infection Control Nurses' Association was formed and worldwide there are now more than thirty-five countries employing infection control nurses.

In 1980, a national survey of Infection in Hospitals was undertaken to investigate the prevalence of hospital acquired infection (HAI) in the UK (Meers et al 1981). A total of 18,163 patients were surveyed in 43 hospitals and of these 19.1% patients were infected and 9.2% of the infections were HAI. Overall, Gram positive organisms were responsible for 30.4% of HAI whereas Gram negative organisms were responsible for 60.1% of HAI. Organisms regarded as the most common cause of infection were identified and are listed in Table 1.

Table 1.

1980 National Survey of Infection in Hospitals

Organisms regarded as most common cause of infection	
Staph . aureus	17.6%
Strep. pneumoniae	1.8%
E. coli	26.1%
Proteus spp.	11.2%
Klebsiella spp.	7.2%
Ps. aeruginosa	7.0%
H. influenza	1.7%
Bacteroides spp.	1.7%
Candida	4.1%
(Viral infections)	0.8%
Salmonella spp.	0.1%

Reference: Meers et al 1981

1.1.3 History of the use of Chemical Disinfectants

Whilst the intelligent use of germicidal agents based on scientific principles covers a period of little more than a century, some empirical practices of a useful nature have been employed for many hundreds or even thousands of years. The Hebrews, Greeks and Romans employed sulphur for fumigation purposes as a religious rite and burnt aromatic woods in the streets to ward off the plague and leprosy. Persian law directed that drinking water should be kept in bright copper vessels and silver was also used for the same purpose. In the Middle Ages, burning of sulphur and of certain woods, notably juniper and cedar and the restricted combustion of other substances was practised as a means of combat ing the spread of bubonic plague. It is now known that the vapours and gases obtained from the processes often contained large amounts of formaldehyde.

It is clear that initially chemical germicides were used as deodorants and antiseptics and it was some time later before they were employed as disinfectants. Reddish (1957) reviewed the history of the use of chemical agents and the following is extracted from his book.

Solutions of chlorine compounds and carbolic acid were first used in the 19th Century. Since foul odours were often associated with disease, the first germicides were those which were effective deodorants.

In 1825, Labarraque recommended the use of chlorinated soda solution in the treatment of infected wounds and also recommended it for general disinfection. In 1827, Alcock introduced the chlorinated soda solution into England and recommended its use for the purification of drinking water.

At this time it was also used in France for disinfecting hands and as an important adjunct in the treatment of hospital gangrene. In 1861, chlorine was used by Semmelweis for cleansing hands as an aid in preventing puerperal fever (see page 17). Such applications were primarily for the purpose of cleansing and deodorizing and it was only when Koch reported on the bactericidal properties of sodium hypochlorite in 1881 that it became clear that sodium hypochlorite could destroy infective microbes.

In 1886 the Committee on Disinfectants of the American Public Health Association reported favourably on the use of sodium hypochlorite solutions as disinfectants.

Probably the first reference to the use of chlorine solutions for disinfecting equipment in the food industry was made by Mohler in 1912 when calcium hypochlorite was used as a disinfecting agent for milk bottles. Since that time, chlorine has been widely used as a disinfectant in the food industry.

The history of carbolic acid (phenol) followed a similar pattern. It was first used as a deodorant on rubbish and in sewage to prevent foul odour. Although carbolic acid was discovered in 1834 it was several years later before it was used in the treatment of wounds. Phenol has been extensively studied ever since Lister promoted its use as a germicide in 1867. Originally phenol and its derivates were obtained from coal tar but a newer source of phenolic material suitable for use in the formulation of disinfectants is petroleum. In 1906 Bechhold and Ehrlich observed that the antibacterial effectiveness of phenols could be potentiated by their halogenation.

Pine oil emulsions were first recommended in 1915 by Stevenson of the United States Public Health Service who advocated their use as general disinfectants. The pine oil itself is obtained from waste pinewood and is blended with emulsifying material in order to produce pine oil disinfectant.

The quaternary ammonium compounds (quats) were first introduced as general and medical disinfectants by Domagk in 1935. They have been particularly favoured for use in the food industry due to the fact that they are odourless, colourless and non-toxic when used in recommended concentrations.

Currently, all of the chemicals listed above are still employed in some form as disinfectants. Whilst research to find novel chemicals which will prove to be effective disinfectants continues, much disinfectant research is focused on new methods of applying existing disinfectants, for example, sustained-activity systems on surfaces and sustained-release systems in cloths and in toilets.

1.2 The Inanimate Environment and Infection

In order to assess the potential infection risks presented by our inanimate environment, it is necessary to define the relevant terminology.

1.2.1 Sources and Reservoirs of Disease

If pathogenic (ie potentially disease-producing) bacteria are to retain their capacity to cause disease, they must have a suitable environment in which to maintain themselves. The terms 'sources' and 'reservoirs' are used and are defined differently by differing authors. The following hospital definitions are quoted from Lowbury et al 1981. "A 'source' of hospital infection is a place where pathogenic micro-organisms are growing or have grown and from which they can be transmitted to patients (eg an infected wound, the nose or faeces of a carrier, contaminated food, contaminated solutions). A 'reservoir' is a place where pathogens can survive outside the body and from which they could be transferred, directly or indirectly, to patients (eg static equipment, furniture, floors)". Lowbury et al state that the terms are sometimes used interchangeably. In a paper on sepsis due to Gram-negative bacteria in hospitals, Parker (1971) described the existence of "free-living" Gram negative organisms from environmental sources. These organisms, whilst capable of causing infection, are usually found in soil, water and other damp places unlike the normal gut flora of man. By contrast, the authors of standard texts in food microbiology (eg Hobbs and Roberts 1987) use the terms sources and reservoirs interchangeably to describe "animal and human reservoirs or sources of food-poisoning bacteria".

1.2.2 Transmission of Infectious Microbes

Disease transmission refers to the method of transfer of infectious microbes from a source or reservoir to the host. Transmission may occur by contact, through the air or by means of a vector. This thesis is particularly concerned with disease transmission by contact.

Contact as described by Boyd and Hoerl (1986) refers to the spread of microbes following either direct or indirect contact with the microbial source. Direct contact refers to the transmission of microbes by close personal association. Handshaking, kissing, sneezing, coughing and sexual contact represent the most usual methods of direct transfer. Direct contact may also be responsible for the endogenous or self-infections which are caused by the patient's own microbial flora and are a frequent cause of HAI. For patients undergoing examination or treatment, indigenous microbial species may be transferred from one body site to another.

Indirect transmission involves the transfer of infectious microbes on various vehicles or disseminators such as food, dust, water, pharmaceuticals or fomites (inanimate objects other than food or water) or on the surface of the hands. The inanimate objects may be contaminated from an animate source or inanimate reservoir. In some cases it is not possible to define precisely what is a reservoir and what is a vehicle of transmission, for example, organisms may survive and grow on a cleaning cloth which will also act as a vehicle for transmission. Contaminated objects are a method of disease transmission in hospitals. Catheters, needles and other objects may be contaminated and when these devices come into contact with the patient they may initiate disease.

In food processing plants, catering establishments and domestic kitchens etc., large numbers of organisms from contaminated food can be deposited onto surfaces such as chopping boards and preparation surfaces and then transferred to other foods which subsequently come into contact with that surface.

In a previous study of environmental sites and surfaces in the domestic environment (Scott 1981), three areas of risk were identified, namely 'reservoirs', 'reservoir/disseminators' and 'contact sites'. The term 'reservoir' was defined as wet sites such as sinks, drainers and wastetraps where free-living organisms can survive and multiply. The term 'reservoir/disseminator' referred to wet or damp objects comprising wet cleaning utensils such as cloths and mops which act not only as reservoirs but also as disseminators of contamination in the environment when improperly handled. The term 'contact site' referred to hard surfaces which come into direct or indirect contact with foodstuffs (eg chopping boards) or which are directly contacted by the hands (eg taps, handles, work preparation surfaces). These terms apply equally well to other environments such as catering establishments and hospital wards. For hospital wards, contact surfaces would include medical equipment and patient care equipment.

1.2.3 Factors which affect the transfer and susceptibility to infection

Where indirect transfer of microbial contamination is concerned, a number of factors will affect and determine the risks of an infection outbreak.

The majority of primary pathogens are extremely sensitive to drying and die rapidly outside the body, although there are exceptions such as the TB bacillus and the anthrax bacillus. Organisms such as Salmonella and Staphylococcus aureus, however, not only survive but grow rapidly if transferred to food. Many so-called opportunist pathogens can also survive outside the body, for example, Ps. aeruginosa will survive and grow under moist conditions apparently devoid of nutrients (Baird et al 1976).

Other inter-related factors which will determine the risk of infection include resistance of the patient, the infective dose and portal of entry. Host resistance or immunity varies greatly between people and although a number of contributory factors can be identified, the variability is still largely unexplained. Resistance may be determined by age, for example, newborn infants are particularly susceptible to gastroenteritis and old people are especially liable to respiratory disease. General health factors which may also produce an increase in susceptibility to infection include:

i) damage to skin and mucous membranes

ii) dietary deficiency in vitamins A, C and proteins

iii) excessive alcohol consumption

iv) fatigue

v) treatment involving radiotherapy or immunosuppressive drugs

The number of organisms required to cause disease is also an important factor although this must be considered in relation to portal of entry and host resistance. In a study on experimental aspects of

local infections, Marples (1976) found that the inoculum of Staph. aureus required to infect traumatised and occluded skin to be as little as 20 viable cells compared with many thousands required to cause infection in a clean open wound. In food poisoning, the numbers of ingested salmonella may be as many as 10^6 or as little as 10^2 or less (Gill et al 1983; Greenwood and Hooper 1983; D'Aoust 1985)

1.3 The Working Environment - Situations of Increased Risk

Microbes are ubiquitous in the general environment in water, air, soil, dust and on vegetation etc. They also occur within our living and working environment in the air, in water supplies, on floors, walls and all surfaces etc. Generally, the presence of microbes in the environment presents no threat but there are certain work situations where the risks of infection are relatively much higher and from which pathogens must therefore be excluded as far as possible. In highly sensitive situations sterile or near sterile conditions may be required.

The three major work situations which require control of environmental microbial contamination are the manufacture and preparation of foods, the clinical environment and the manufacture and preparation of pharmaceutical products and cosmetics. Within these workplaces, control of contamination may be confined to specific sites and surfaces or it may extend to the whole working area.

1.3.1 Food Manufacture and Preparation

Whenever food is manufactured or prepared, hygiene procedures are required to prevent gross contamination. It is recognised that food-borne disease, as discussed on page 13 has a considerable economic and social impact on the public sector, on the food industry and on society in general including the affected person and his or her family. In a survey of foodborne illness, Sockett (1989) estimated the average investigative cost of a food poisoning case to be £104. In 1986 the health care costs per patient treated for salmonella food infection

were reported at £376 (Gilbert 1986). In 1985, the total health care bill for salmonella infection was estimated at £4.5 million (Gilbert 1986). Sockett (1989) provided some examples (Table 2) of the total costs of various food poisoning outbreaks dividing the total costs between public sector cost and society cost. Public sector costs fall on services directly involved in the treatment of the patient and investigation of the incident. Costs to the food industry occur when a particular product, retail outlet or restaurant is associated with an outbreak. Such incidents may result in loss of business, plant closure and loss of jobs. Social costs represent both the effects of illness on the affected individual and his or her family as well as lost production from sickness absence. The costs associated with outbreaks due to manufactured foods tend to be considerably higher than those associated with non-manufactured foods. This is largely because losses resulting from recall and destruction of product, capital expenditure on equipment cleaning or replacement and loss of business must be taken into account. Public sector costs although relatively small are significant and represent a considerable drain on local resources.

In addition to these tangible costs, general discomfort and in extreme cases death, loss of leisure, loss of housewives' time and loss of schooling have significant if difficult to value effects, on the individual. The most common contributory factors identified for food poisoning are listed on page 16 and include lack of proper temperature control and poor storage of foodstuffs. Although cross-contamination via inanimate surfaces and via hands appears quite low on the list of contributory

Table 2. Food Poisoning Costs

Year	Agent	Food	Public Sector Cost(£)	Society Cost (£)	Total (£)
Manufactured Food					
1985	S.ealing(76)	Milk Powder	163.012	49,124,000	49,287,012
Non-manufactured Food					
1986	Campylobacter (53)	Poultry	7,129	23,984	31,113

Number of recorded cases in brackets

Reference: Sockett (1989)

factors, experts believe that cross-contamination plays a much greater part than is indicated (Gilbert 1986, De Wit et al 1979, van Schothurst et al 1979).

The manufacture or preparation of food for sale is subject to legislation (Food Hygiene (General) Regulations 1970) the purpose of which is to ensure a suitable environment for food production. These regulations require that food premises conform to a suitable standard of construction and cleanliness, that food is handled hygienically, that employees adopt suitable standards of cleanliness and that suitable facilities are made available to them. With regard to environmental contamination, the following points are stated in the regulations:

1. Premises - No food handling business can be permitted in premises which are insanitary or where food is exposed to the risk of contamination as a result of the poor condition or situation of the premises. Every food room should be kept clean and in good repair to prevent, as far as is reasonably practical, any risk of infection.
2. Equipment - Articles or equipment in contact with food must be kept clean and in good repair.
3. Facilities and amenities - Adequate wash-hand basins must be readily accessible to food handlers; soap, nail brushes and suitable drying facilities must be provided. Sanitary conveniences of a suitable standard must be provided.

The Food Hygiene Regulations do not specify the sites and surfaces within the workplace which are most likely to represent a hazard, but some valuable information can be obtained from the literature.

Mendes et al (1978) carried out a bacterial survey of some 5,000 sampling positions in 100 kitchens of a variety of premises including restaurants, hotels, public houses, industrial, hospitals and schools. Kitchens were divided into 5 areas relating to different tasks as follows:

1. Storage and initial preparation
2. Final preparation
3. Cooking
4. Serving
5. Washing up

The surfaces which were sampled by swabbing included sinks, taps, overflows, worktops, chopping boards, meat slicers and handles etc. The results were presented as total viable counts together with bacterial identification. The results indicated that overall a high percentage (59%) of the bacterial species isolated were of faecal origin and that the pattern of contamination varied according to the task being performed. For example, initial food preparation areas were more heavily contaminated with coliforms than cooking and servicing areas, indicating contamination from raw incoming food. Washing-up areas were also heavily contaminated with coliforms which probably originated from the equipment used to prepare raw foods.

In the initial preparation areas, surfaces such as chopping boards, door handles, sinks and taps were found to have the highest coliform counts (20% of sites gave counts of greater than $10^3/cm^2$). In the final preparation areas food processing equipment and surfaces were less often contaminated with coliforms than initial preparation areas but coliforms were at some time present

on food preparation surfaces and equipment such as meat slicers and can-openers together with sites such as handles and tops. In the cooking areas, contamination was less than in preparation areas but even here 33% of worktop surfaces were found to be contaminated with coliforms. In the servicing area contamination with coliforms was less than in the other areas and yet 16% of worktop surfaces and 30% of hot cupboard handles were contaminated with coliforms and counts of greater than $10^3/cm^2$ were recorded. The authors commented that the occurrence of coliforms was unexpected in the servicing area and that such contamination must have reached this area on hands and cloths. In the domestic kitchen the quality of food hygiene practice is dependent upon the level of "education" of the consumer. Such education may be acquired in school, at evening classes and from the media etc. but there is no single route by which information on food hygiene may be communicated to the population. The practice of food hygiene skills in the home is essential in order to prevent food borne disease. There is data, as shown in Table 3, to suggest that more food poisoning outbreaks occur in the home than in public premises.

Table 3.

Common locations of outbreaks of food poisoning 1986-1988

	1986	1987	1988	Total
Private houses:	326	324	320	970
Other locations:				592
including				
restaurants/hotels				
receptions	71	92	90	253
hospitals	33	26	21	80
institutions	19	13	29	61
canteens	9	4	14	27
schools	8	3	6	17
shops	4	13	11	28
farms	2	-	3	5

References: PHLS unpublished & Sprenger 1989

A bacterial survey of the domestic environment similar to that of Mendes and Lynch's (1978) survey of public premises was used to assess contamination risks according to sites. A survey of some 70 sites in over 200 homes involving sampling for contamination levels and the occurrence of potentially pathogenic species was published by Scott (1981). Sampling was carried out mainly in the kitchen, bathroom and toilet but also in the general living areas. In particular, the results of the survey indicated that the risks associated with the general environment differ considerably for different sites or groups of sites. Those sites most frequently contaminated and therefore most likely to be associated with infection transfer can be reasonably assessed if the sites are grouped under the headings of 'reservoirs', 'reservoir/disseminators' and 'contact surfaces' as defined on page 26 . Classification of sites and surfaces in the home environment using this approach is given in Table 4 . In order to assess risk, the frequency of occurrence of potentially pathogenic species was considered together with the occurrence of high contamination levels. The results as illustrated in Fig 2 indicated that wet/reservoir and reservoir/ disseminator sites were most commonly associated with high counts and the presence of organisms of enteric origin. In the kitchen, raw food is probably the main source by which contamination is continually introduced but the sink, wastetrap and surrounding areas act as reservoirs which harbour and encourage proliferation of enterobacteria.

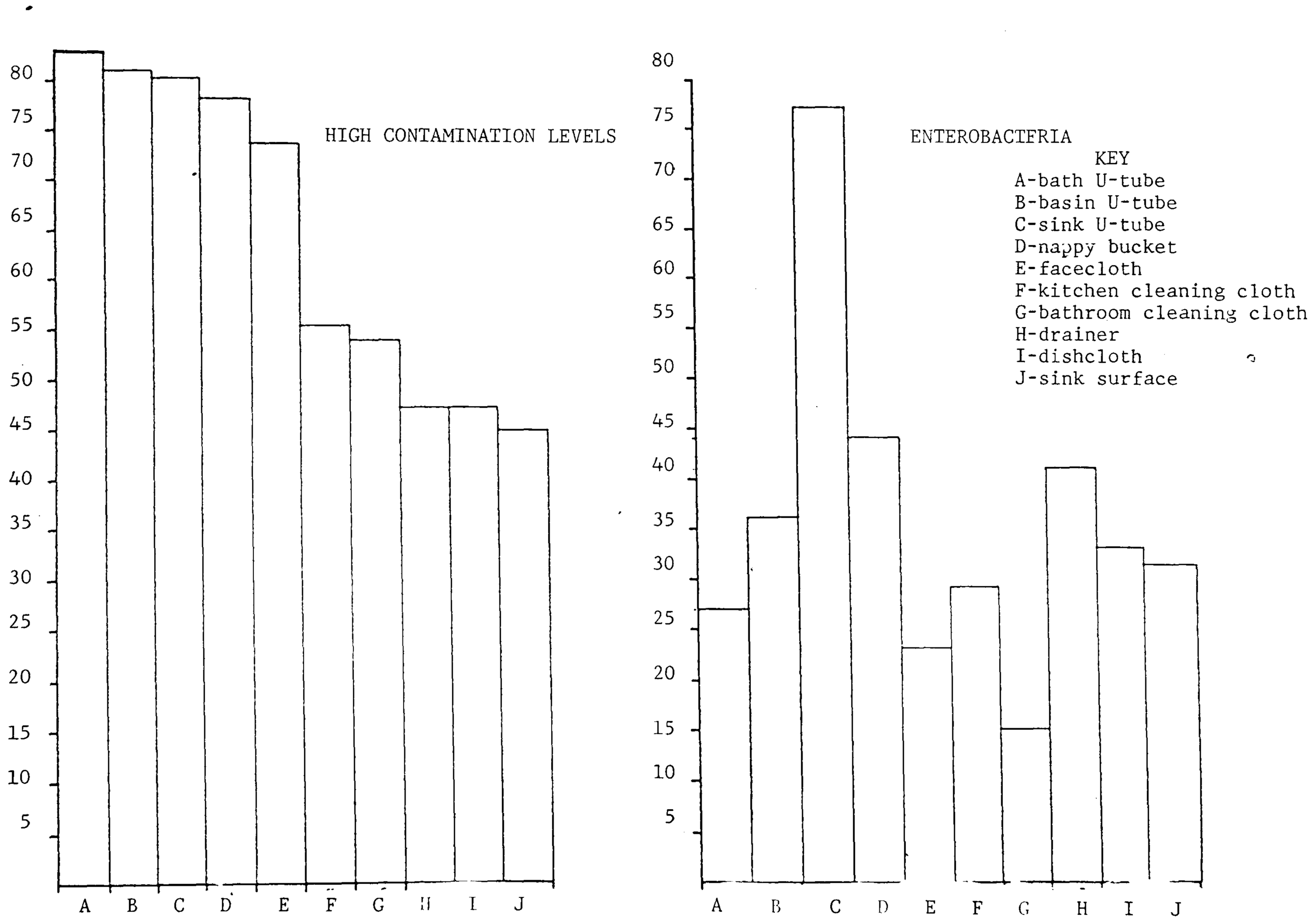
Reservoir/disseminators such as dishcloths and other wet cleaning utensils were also found to be contaminated with large numbers of organisms including enterobacteria.

Table 4 Classification of sites and surfaces in the home

Reservoirs of contamination	:	toilets and nappy buckets sink and sink drainer areas wastetraps
Reservoir/disseminators	:	wet cleaning utensils such as cloths and mops
Contact surfaces	:	food preparation surfaces, taps handles, toilet seat

Ref. Scott 1981

Fig 2 The percentage frequency occurrence of high contamination levels and enterobacteria at selected sites



Reference: Scott 1981

In the bathroom and toilet the same pattern of contamination was found. Although enteropathogenic organisms probably originate from the toilet and directly from people using the bathroom and toilet, the results indicated that baths, basins and cleaning cloths may harbour and encourage proliferation of permanent reservoirs of these organisms. Enterobacteria were quite often isolated from toilets (approximately 30% of toilet water samples) but the frequency of high counts was less than at other wet sites.

In addition, although the occurrence of high counts was relatively less frequent at food and hand contact sites, small numbers of potentially pathogenic organisms were isolated from a total of 49% of all food contact and 28% of all other hand contact sites examined in this survey.

1.3.2 The Clinical Environment

The hospital is a unique environment which contains not only routine areas such as kitchens, bathrooms and toilets but also areas of advanced technology such as operating theatres and intensive care units. Within this environment are the patients, who as a group are particularly susceptible to infection as discussed on page 16.

Currently, as detailed on page 19 , in the United Kingdom, it is estimated that approximately 9% of patients acquire a hospital infection.

Factors which contribute to the financial costs of HAI include increased length of hospital stay, additional use of laboratory facilities and the requirement for antimicrobial drugs etc. An attempt was made to cost

HAI in the United States (Daschner 1982) and based on HAI figures for 34 million admissions, the cost at that time was estimated at a billion dollars. This costing did not include indirect costs such as absence from work. Girard et al (1983) reported that the costs of HAI in a neonatal unit increased the total hospitalisation costs by 32%. Neither of these examples take into account the additional physical and emotional distress and suffering of the patient and his or her family as a result of HAI.

There are many references in the literature concerning outbreaks of HAI which implicate an environmental site or surface as either the reservoir or source of the infecting agent. In a paper entitled 'The hospital environment as a source of septic infection', Parker (1978) discussed the importance of contaminated environmental sites either as a temporary reservoir or as an independent source of infection. As vehicles in person-to-person spread, Parker included both inanimate surfaces and objects although he named hands as the most important vehicle. Further, he divided contaminated inanimate surfaces into those in the immediate environment facilitating close contact and those in the 'general' hospital environment. Hospital environmental sites and surfaces can also be divided into three categories based on patient risk, as defined by Lowbury et al (1975). In this scheme, surfaces are assigned a high, intermediate or low risk category as shown in Table 5 .

Specific examples of environmental sites implicated as the source or reservoir of infection in cited infection outbreaks which can be grouped under the headings of

Table 5

High, intermediate and low risk environmental sites and surfaces in hospitals

Category	Items include
High risk - surfaces of equipment which comes into close contact with a break in the skin or mucous membrane, or is introduced into a sterile body area	surgical instruments syringes and needles catheters, etc. endoscopes
Intermediate risk - surfaces of equipment which comes into contact with intact skin or mucous membrane	respiratory equipment, thermometers, trolley tops, bedpans, wash bowls, urinals etc.
Low risk - equipment or parts of the environment not in close contact with the patient or his immediate surroundings	floors, walls, work surfaces, baths, toilets, washbasins etc.

Reference: Lowbury et al 1975

'reservoirs', 'reservoir/disseminators' and 'contact surfaces' as defined on page 26 are summarized in Table 6 and are as follows:-

a. Reservoirs of Contamination

Kohn (1967) describes a study of sinks and wastetraps as both a reservoir of contamination and possible source of Pseudomonas aeruginosa cross-infection in burns units. It was found that patients' raw burns were colonized or infected by strains which had been isolated from the washbasin traps prior to the patients' admission. In a study prompted by concern over high rates of infection in clean wounds, Thomas et al (1972) found that operating theatre plumbing supplies and outlets were heavily contaminated with Gram negative bacilli. The authors demonstrated splashback from sink plugholes using blotting paper and blue dye from which they concluded that splashback of bacterial contamination could be sufficient to contaminate a surgeon's gown or to contaminate and colonize scrubbing-up taps. Teres et al (1973) monitored pseudomonas pyocine types from 640 patients and their environment in a respiratory/surgical intensive-ther apy unit. Results showed that forty of fifty-six sputum/throat isolates were hospital acquired. Pyocine types 1 and 10 were the 'resident' strains and both types were regularly cultured from only one environmental source, namely sinks. The authors suggested that the sinks provided reservoirs of pseudomonas infection and that the hands of personnel transmitted the infection. In a study of an outbreak of enteritis and septicaemia caused by S. enteritidis in a nephrology unit (Lockyer et al 1980), a contaminated sink and refrigerator in

Table 6. Contaminated environmental sites and surfaces in hospitals

Reference	Surface	Organism involved
a) Reservoirs of contamination		
Millership et al 1986	Sinks	Gram negative bacilli
Zimakoff et al 1983	Sinks	Ps. aeruginosa
Fujita et al 1983	Sinks	Ps. aeruginosa A. anitratus
Casewell 1981	Waterbath	Ps. aeruginosa
Lockyer et al 1980	Sinks	
Brown et al 1977	Sinks	Ps. aeruginosa
Teres et al 1973	Sinks	Ps. aeruginosa
Thomas et al 1972	Sinks & wastetraps	Gram negative bacilli
Kohn 1967	Sinks, wastetraps & basins	Ps. aeruginosa
b) Reservoir/disseminators of contamination		
Zimakoff et al 1983	Wet brush & cloth	Ps. aeruginosa
Thomas et al 1972	Floor scrubbing machine	Ps. aeruginosa
c) Contact sites		
Zimakoff et al 1983	Soap, bath toys, table top	Ps. aeruginosa
Lockyer et al 1980	Refrigerator door lining	S. enteritidis
Taylor et al 1979	Kitchen work-surfaces, door hand plates, cot rails, light switch	Gram negative bacilli
Thomas et al 1972	Taps	Gram negative bacilli

the ward kitchen were identified as the most likely source of infection.

In another study of Ps. aeruginosa in a cystic fibrosis unit (Zimakoff et al 1983), samples were taken from patients staff and environmental sites. Ps. aeruginosa was isolated from 51% of patients and strains of the same epidemiological types were isolated from sinks and other environmental sites. The authors demonstrated that splashback from sinks during handwashing was sufficient to cause dissemination of contamination.

b. Reservoirs/disseminators of contamination

Whilst the use of 'multiple-use' wet cleaning items is nowadays discouraged in hospitals in the UK, wet cleaning equipment has been found to be a cause for concern (Colquitt and Maurer, 1969; Baird et al 1976).

In their operating theatre study, Thomas et al (1972) found the floor-scrubbing machine to be heavily contaminated with Ps. aeruginosa and other Gram negative bacilli. Experiments with this machine demonstrated that it was capable of causing gross contamination of the theatre suite.

c. Contact sites

Within the hospital environment there are many references to contaminated hand contact sites. Taylor et al (1979) found many hand contact sites in a children's hospital to be grossly contaminated with Gram negative bacilli. Sites included worksurfaces and door handplates in the milk kitchen and cot rails and light switches in the ward cubicles.

In an epidemiological study of Ps. aeruginosa infection

in a cystic fibrosis clinic (Zimakoff et al 1983) items such as soapbars, toys and tabletops were found to be contaminated. It appeared that CF patients contaminated the environment as a result of coughing. Experiments showed that Ps. aeruginosa could survive after drying on a polyvinyl surface for at least five days when present in sputum

During the 1960's and 1970's there was a trend towards routine sampling programmes in hospitals to determine general levels and to define acceptable limits for contamination. More recent experience suggests little correlation between overall levels of microbial contamination in the hospital environment and the incidence of infection and this practice or policy has now been abandoned. The environmental studies referred to in this section were initiated as a result of infection outbreaks. This approach of 'sampling by objectives' is now considered to be the most worthwhile approach to hospital sampling programmes because it concentrates on areas most likely to be associated with infection and where infection control measures are most likely to be effective.

1.3.3 Pharmaceutical manufacture and preparation

During the manufacture and preparation of pharmaceutical (and cosmetic) products, both in a hospital or industrial environment, some degree of microbial contamination is likely unless products are subjected to a sterilisation process. Additionally, products are vulnerable to contamination during use as well as during manufacture.

The potential hazard of microbial contamination of pharmaceutical products is twofold. Firstly, contamination may cause product spoilage resulting in chemical modification. Secondly, contamination may also constitute a direct hazard to the patient, for example in parenterals and eye solutions, although it must not be assumed that administration of pathogenic species to a patient will necessarily cause infection. As with infections in general (page 26), the infection risk from contaminated pharmaceuticals depends on four factors, namely, the type of organism, the infective dose, host resistance to infection and route of administration. Generally the risk of infection will be less for a product given orally or applied to intact skin compared with a product used for treatment of damaged skin or mucous membrane, or a damaged eye. For products which are introduced into normally sterile areas of the body, the potential risks are considerable.

Recently, Denyer and Baird (1990) reviewed infections associated with contaminated pharmaceutical products. Infections are reviewed according to the following routes of administration: oral medicines, topical preparations, respiratory products, disinfectants, ophthalmic preparations, irrigating fluids and dialysis fluids and finally, injections. Types of infection range from outbreaks of gastrointestinal infection associated with oral preparations contaminated with Salmonella species to skin, urinary tract and septicaemia infections associated with topical preparations contaminated with Klebsiella pneumoniae and Pseudomonas aeruginosa and respiratory tract infections caused by aerosols contaminated with Klebsiella species and Serratia marcescens.

The decision as to whether or not a product should be sterilised is assessed according to the degree of risk to the patient, for example, products such as injections are sterilised in order to prevent the direct introduction of contamination into the bloodstream etc. Over the years, the range of products now required to be sterile has increased to include formulations such as eye preparations, some topical solutions and more recently, contact lens solutions. However, surveys have shown that a wide range of products may be contaminated, including intravenous fluids, injections, vaccines, antibiotics, creams, handcreams, powders, tablets, mixtures and disinfectants. Table 7 gives a list of surveys of microbial contamination of pharmaceutical and cosmetic products up to 1978 (Denyer and Baird 1990). Out of a total of 6,764 samples examined, about 27% were found to contain detectable contamination, although rates varied from 2 to 80% according to product type. Of the 6,000 or more samples where bacterial isolates were identified, the majority were found to be Gram-positive bacilli and micrococci and generally regarded as non-pathogenic. Of the species regarded as potentially pathogenic, Pseudomonas aeruginosa and other pseudomonads were most frequently isolated.

The frequency of heavy contamination (greater than 10^5/g or ml) has been found to be related to water availability in the product (PHLS Survey Anon 1971 a), so that aqueous products are more likely to be heavily contaminated than dry products.

Table 7 Survey of microbial quality of pharmaceuticals, toiletries and cosmetics

Reference		Type of product	Number investigated	Number contaminated	
Baker	1959	toiletries	5	2	(40%)
Kallings et al	1966	pharmaceuticals	134	91	(68%)
Ulrich	1968	pharmaceuticals	696	535	(76%)
Hirsch	1969	pharmaceuticals	57	47	(82%)
Wolven & Levenstein	1969	cosmetics	250	61	(24%)
Dunnigan & Evans	1970	cosmetics	169	33	(20%)
Anon	1971a	pharmaceuticals	1220	390	(31%)
Beveridge & Hope	1971	pharmaceuticals	138	58	(42%)
Robinson	1971	pharmaceuticals	279	85	(30%)
Wolven & Levenstein	1972	cosmetics	228	8	(3%)
Ahearn et al	1973	cosmetics	200	3	(2%)
Baird et al	1976	pharmaceuticals	499	46	(9%)
Awad	1977	pharmaceuticals	911	109	(13%)
Awad	1977	pharmaceuticals	247	79	(32%)
Awad	1977	pharmaceuticals	110	31	(28%)
Awad	1977	pharmaceuticals	1462	184	(13%)
Baird	1977	cosmetics	147	48	(32%)
Wallhauser	1978	pharmaceuticals	17	12	(70%)
Total			6764	1814	(27%)

Reference: Denyer & Baird 1990

Pharmaceuticals are subject to legislative requirements regarding microbial limits. The importance of microbiological control of non-sterile products has gained recognition over the past two decades. Such products must now comply with the appropriate standards. Control of contamination is brought about mainly by the control of raw materials, by Good Manufacturing Practice (Anon 1983), by product formulation and by the inclusion of preservatives.

A part of GMP is a consideration of the importance of environmental contamination. Within the pharmaceutical manufacturing environment, sites and surfaces most likely to represent a cross contamination hazard are similar to those found in food manufacture and preparation, namely the 'reservoirs', 'reservoir/disseminators' and 'contact sites' (page 26). In the case of pharmaceutical manufacture, contact sites are those surfaces in contact with non-sterile products as well as the hand contact sites. Whilst there is no literature reference to a single comprehensive survey of pharmaceutical manufacturing environments, a study published by Baird et al (1976) gives an indication of the likely problem areas. The environment of eight hospital pharmacies and the preparations made in these pharmacies were examined for Pseudomonas aeruginosa. This organism was found widely distributed in the pharmacies at moist sites such as sinks, drains, draining boards and taps, as well as in cleaning equipment which had been stored wet. The organism was isolated from 9% of preparations and in 11 instances, strains of Ps.aeruginosa from the preparations bore a close resemblance to strains previously found in the pharmacy

environments. Subsequently, an environmental contamination control programme was introduced into two of the hospital pharmacies and included the use of sterilising traps for the sink U-bend, daily swabbing with dilute sodium hypochlorite solution and the use of disposable cloths etc. As a result, isolation of Ps. aeruginosa from the environment was reduced from 18% to 2% and from 21% to 3% in the two pharmacies respectively. (Baird et al 1977). In addition, the overall bacteriological quality of the pharmaceutical preparations was found to have improved. It was also found that products prepared under controlled environmental conditions were less likely to be contaminated with Pseudomonas in comparison with those prepared in pharmacies without environmental controls.

1.4 The role of disinfection and other control measures in the prevention of the transmission of infection

Manufacturing industry (food and pharmaceutical) and hospitals have programmes in place to prevent or reduce the contamination of products or the spread of infection to patients. Whilst such programmes have different names depending on whether they are designed for food and pharmaceutical manufacture and preparation or for hospitals, in essence they are founded upon very similar principles. In hospitals such programmes are entitled 'hospital infection control policies' whereas in industry the terms 'cleaning programmes', 'sanitation programmes', 'line sanitation' and 'quality assurance' etc. are in use. Generally, hospital infection control policies are directed along four main lines as follows:

1. Eradication of potential sources/reservoirs of infection by:
   a. sterilization and/or disinfection before use of all 'critical' items of medical or surgical equipment (including IV fluids etc)
   b. sterilization and/or disinfection after use of material and/or equipment likely to be contaminated with infected material (eg bedpans etc.)
2. Blocking routes whereby organisms are transferred to patient by:
   a. aseptic or hygienic surgical, dressing and general nursing techniques

b. isolation and barrier nursing of susceptible patients

3. Enhancing patients' resistance to infection by:

a. careful handling of tissues and removal of slough/ foreign bodies during surgery

b. antibiotic prophylaxis and treatment

4. Training of hospital staff

Successful control of infection depends on the extent and efficiency with which the above procedures are implemented.

This same policy could be applied directly to the food and pharmaceutical industry simply by substituting the clinical terms as shown below:

1. eradication of potential sources/reservoirs of contamination

a. sterilization and/or disinfection before use of all 'critical' items of equipment (eg worksurfaces, packaging materials)

b. sterilization and/or disinfection after use of equipment likely to be contaminated with infected material (eg chopping boards, knives, work surfaces, on-line equipment, cleaning items)

2. to block routes whereby organisms are transferred to food or product

a. aseptic or hygienic working techniques

b. preparation of sensitive products in isolation (eg inside laminar flow cabinets)

Separation of work areas in kitchens so that raw foods are handled separately to cooked foods

3. Enhance hygiene of foodstuff or product

   a. quality assurance programmes to ensure quality of raw materials. Prevent objects and foreign bodies falling into foodstuffs and products on the production line

   b. terminal heat treatments of foods (eg canning) or products

4. Training of staff

1.4.1 Sterilization and Disinfection

In achieving the first two principles of the above policies, the use of sterilization and disinfection procedures play an important part.

Sterilization is defined as "the total removal of all viable organisms including vegetative bacteria, spores, viruses, fungi, etc." and may be achieved by the following processes:

1. Dry heat - 150-160° for 1 hour
2. Moist heat - 115-116° for 30 minutes or equivalent
3. Irradiation
4. Ethylene Oxide
5. Low temperature steam and formaldehyde
6. Filtration (fluids only)

Disinfection is defined as the "destruction or removal of harmful bacteria (but not spores) to a level not normally harmful to health or to the quality of perishable goods". Removal or destruction of harmful bacteria may be achieved by:

1. soap and water cleaning
2. drying and/or application of heat
3. chemical disinfectants

It should be noted that with regard to the definition of disinfection, the levels of bacteria not normally harmful to health etc. will vary from one situation to another.

Soap and water cleaning will remove contaminated material from a surface but will not necessarily destroy or remove residual bacteria left on the surface.

The maintenance of dry conditions plays an important part in controlling the survival and spread of organisms on inanimate surfaces. Heat is generally regarded as the most reliable method of disinfection. Heat treatment at 65°C for 10 minutes (or equivalent) will destroy most vegetative organisms.

Chemical disinfectants are recommended in situations where the application of heat is neither convenient nor possible, for example, delicate heat sensitive instruments, large work preparation surfaces, floors etc. Within hospitals, the list of chemical disinfectants available includes phenolics, varius chlorine preparations, iodine, gluteraldehyde, alcohols and quaternaries and diguanides. Outside of the hospital, a generally more limited list of chemical types is available for industrial, catering and domestic use and includes hypochlorites, phenolics, quaternaries and quaternary/chlorhexidine.

With regards to the properties of chemical disinfectants, none of the formulations represent the 'ideal disinfectant' and each has particular advantages and disadvantages.

One of the most important factors which affects the suitability of any particular formulation is its antimicrobial activity against a broad spectrum of

vegetative bacterial cells and spores, fungi and viruses. Other factors affecting the activity of different chemical types must also be considered and include inactivation by organic matter, surfactants and hard water. Further considerations include smell, stability in solution, corrosiveness, staining, wetting power, irritability and effect upon the environment. The properties of the various antimicrobial agents used in disinfectant formulations are reviewed by Hugo and Russell (1987) and Russell, Hugo and Ayliffe (1982)

1.5 Aims and Objectives

A survey of the literature as outlined in this introduction reveals that general environmental sites such as sinks and sinktraps, worksurfaces and cleaning equipment are quite frequently reported as likely reservoirs and/or sources of contamination/infection in the food and pharmaceutical industry and in hospitals. This indicates that the potential hazard posed by such sites must be properly assessed. In this study, general environmental sites and surfaces were investigated with reference to the food and pharmaceutical industry and hospitals.

As discussed on page 26 general environmental sites likely to present a potential hazard are grouped under three headings, namely reservoirs, reservoir/disseminators and contact sites. The aim of this investigation was to take typical examples of sites in each of the three groups and to study each site in detail in either a laboratory and/or a field situation in order to determine the following:

1. The extent to which bacterial contamination can survive at each site.
2. The risk of transfer of bacterial contamination from each site to potentially more hazardous sites and surfaces.
3. The effectiveness of various disinfection procedures in breaking the chain of transmission of bacterial contamination from each of the sites.

The examples chosen to represent the three risk groups are toilets and sink wastetraps (reservoirs), wet cleaning cloths (reservoir/disseminators) and worksurfaces (contact sites).

As a result of this detailed examination of the survival, transfer and disinfection of environmental contamination, the implications in relation to current methods used in control of infection and contamination in hospitals, in food preparation and pharmaceutical manufacturing environments are considered.

CHAPTER 2

MATERIALS AND METHODS

2.1 Media and solutions

Except where stated otherwise, all culture media were prepared in the laboratory using media bases obtained from Oxoid Ltd (Basingstoke, Herts). Contact plates were prepared weekly and overdried for 1 hour in a lamina flow cabinet prior to use. Quarter strength Ringer solution and normal saline solution (0.9%) were prepared from tablets obtained from Oxoid Ltd.

2.2 Test organisms

Freeze dried cultures were obtained from the National Collection of Type Cultures and included the following:

Pseudomonas aeruginosa 6749 and 6750

Salmonella abony 6017

Escherichia coli 8196

Klebsiella aerogenes 5055

Staphylococcus aureus 4163

These strains will be referred to hereafter as "laboratory strains" or 'ls'.

The following environmental isolates were obtained and are referred to hereafter as "wild type" strains or 'wt'.

Salmonella spp (API 20E profile 6504512)

Escherichia coli (API 20E profile 1044512)

Klebsiella pneumoniae (API 20E profile 5004773, non-motile methyl red negative)

Staphylococcus aureus (Coagulase and DNase positive)

2.3 Reconstitution of freeze dried cultures

NCTC freeze dried cultures were reconstituted in 10ml of nutrient broth and incubated at 30°C for 24 hours.

2.4 Maintenance of stock cultures

Stock cultures of the various test organisms listed above were prepared and maintained on nutrient agar slopes and stored at 4°C. Subcultures were prepared monthly on fresh agar slopes, incubated at 37°C for 18-24 hours and then stored at 4°C.

2.5 Identification of bacterial species

Identification of bacteria isolated from environmental sites was made by observation of colonial characteristics and Gram staining (Jensen method described by Cruikshank et al 1973). Gram negative bacilli were further identified by the API 20E system (API Laboratory Products Ltd, Basingstoke) while presumptive Bacillus cereus and enterococci were identified using egg yolk agar and Slanetz and Bartley agar respectively. Staphylococcus aureus was identified by the DNase Test described by Jacob et al (1964) and/or the Coagulase test. Pseudonomas aeruginosa was confirmed using King's A media.

2.6 Nomenclature

Nomenclature used for Gram negative rods identified by the API system is that described by Edwards and Ewing (1972). Nomenclature for other bacterial types is that described in Bergey's Manual of Determinative Bacteriology (1974).

2.7 Incubation temperature

All contact plates and Petri dishes were incubated for 24 hours at a temperature of 37°. If no growth occurred after this period, samples were incubated for a further 24 hours.

2.8 Pipettes

Throughout this study, 2 sizes of Finn Pipette (supplied by Jencons, Hemel Hempstead) were used, namely 0-50 ul and 1-5 ml, for preparation and dilution of cultures and test agents etc.

2.9 Surface viable counts

Surface viable counts for confirmation of the number of colony forming units per ml of overnight broth culture or test suspension etc were performed using the Miles and Misra methods (Miles et al 1938).

2.10 Preparation of overnight broth cultures

Test organisms taken from stock nutrient agar slopes were inoculated into 10ml of tryptone soya broth. Surface viable counts indicated that an overnight both culture of Gram negative species yielded approximately 10^9 organisms/ml (mean 1.35×10^9, range $2.6 \times 10^8 - 3.4 \times 10^9$) and overnight both cultures of Gram positive species yielded approximately 10^8 organisms/ml (mean 2.2×10^8; range $1 \times 10^7 - 5 \times 10^8$).

2.11 Preparation of test suspensions in broth or water

Werner (1975) described the use of tryptone soya broth to simulate soiled conditions on surfaces and this procedure was adopted for in vitro experiments in this study. To simulate soiled conditions, overnight tryptone soya broth cultures were appropriately diluted in fresh broth to give the required number of organisms/ml.

To simulate clean conditions, cultures were prepared in sterile distilled water as follows. Overnight broth cultures were centrifuged in a Mistral 2L centrifuge at 2,600 r.p.m. for 10 mins. The broth supernatant was discarded and the culture pellet re-suspended in 10ml of sterile distilled water. After a second centrifugation at 2,600 r.p.m. for 10 mins, the water supernatant was discarded and the pellet re-suspended in a further 10 ml of water. This suspension was diluted with sterile distilled water to give the required number of organisms/ml. Suspensions were standardised by the method of Miles and Misra (1938).

2.12 Preparation of test suspensions in water, 5% plasma and 20% plasma

For the purposes of experiments described in chapter 6 to assess disinfectants, the following method was adopted for the preparation of test suspensions. Cells of E. coli (1s), Staph. aureus (1s) and Ps. aeruginosa (1s 6750) were harvested from overnight nutrient agar roux slopes, washed twice by centrifugation (described above) and resuspended in quarter strength Ringers solution (8ml) to give a total count of 10^{10} organisms/ml (confirmed by a surface viable count).

To simulate clean conditions, a series of decimal dilutions in quarter strength Ringers was prepared to give a range from 10^{7} to 10^{3} organisms/ml.

To simulate heavily soiled conditions, a series of dilutions was prepared in quarter strength Ringers

and Human Plasma BP (Immuno Ltd, Sevenoaks, Kent) containing 4.3% w/v plasma protein, to give a range from 10^7 to 10^3 organisms/ml in 20% v/v Plasma.

For E. coli only, a series of dilutions in the range 10^7 to 10^3 organisms/ml in 5% v/v plasma was prepared.

2.13 Worksurfaces models (laminate squares)

A white laminate (formica type) material was chosen to represent a type of worksurface that is commonly encountered.

Contact adhesive was used to back the laminate squares ($20cm^2$) onto stainless steel in order to prevent the laminate from bowing.

2.14 Preparation of laminate squares

Laminate squares were prepared for experimental use by swabbing with 70% alchol. The squares were then immediately placed in assay dishes in a lamina flow cabinet prior to inoculation.

On occasions, there were indications that the laminate squares had become contaminated with Gram positive aerobic spore-bearing organisms. The squares were then soaked for 10 minutes in hypochlorite solution (available chlorine 100 p.p.m.) followed by 2 changes of sterile distilled water (10 minutes each). The squares were then allowed to drain and were swabbed with 70% alcohol.

When not in use, the laminate squares were stored in closed assay dishes.

2.15 Cloths

Cloths used throughout were of the disposable dry-woven type (J cloths) supplied by Paynes Scientific, Slough.

2.16 Preparation of sterile clean cloths

Unused rectangular cloth portions (1260 cm^2) were soaked in 0.9% (normal) saline solution for 10m. Studies by Elworthy and Graham (1969) indicate that prewetting of cloths with saline, thus allowing the saline to fill the capillary network of the cloths, can substantially reduce adsorption of contamination. Following soaking, cloth portions were wrung out, folded and placed in glass Petri dishes. These dishes were then steam sterilised at 121°C for 15m.

2.17 Preparation of sterile soiled cloths

Cloths which had been in use as washing-up cloths in domestic kitchens for 3 days were returned to the laboratory, cut up into rectangular portions (1260 cm^2) packed into Petri dishes and steam sterilised as above. In this way, cloths were obtained for experimentation which were both sterile and naturally soiled from kitchen usage. It was not considered necessary to soak these cloths in normal saline solution.

2.18 Preparation of naturally contaminated cloths

Naturally contaminated cloths were prepared as follows. Volunteers were requested to take home and use the cloths (as described in 2.15) as 'washing-up' cloths etc., in their kitchens for 3 days. Instructions

were given that the cloths were not to be used in combination with chemical disinfectants in order to prevent any carry-over of disinfectants into the test procedure. After 3 days, the cloths were returned in polythene bags to the laboratory for immediate investigation.

2.19 Sampling with contact plates

In order to standardise the technique of contact plate sampling in laboratory studies, contact plates were weighted with 200 grm. weights whilst placed in contact with a surface for 30 seconds. In field studies, contact plates were hand-held in contact for 10 seconds.

Colony counts of greater than 250 per contact plate were recorded as "too numerous to count" (TNTC).

2.20 Rinse method for estimation of total counts in cloths

In order to evaluate the total count of organisms in cloths the following method was employed. At the end of an experiment, cloth portions were immersed in sterile quarter strength Ringer solution (100 ml) in sterile conical flasks (250 ml). The flasks were then shaken on a mechanical shaker for 10 mins. in order to release organisms from the cloths. Surface viable counts were performed on the rinse solution. Results are expressed as the total count per cm^2 of cloth.

2.21 Disinfectant and detergent test agents employed in the disinfection of cloths (Chapter 5)

Test agents employed in Chapter 5 for the disinfection

of cloths comprised a clear soluable phenolic fluid (brand name 'Stericol', Sterling Industrial Ltd, Sheffield), sodium hypochlorite solution containing 10-14% w/v available chlorine (B.D.H. Ltd, Dagenham) and a proprietary anionic green liquid detergent (Waitrose Ltd, Bracknell). Sterile distilled water was used as a control.

Following the use dilutions recommended in the Kelsey-Sykes capacity test (Kelsey and Maurer 1974) for soiled conditions, the phenolic and hypochlorite agents were diluted in sterile distilled water as follows:

	Use-dilution % v/v
Stericol	2%
Sodium hypochlorite	4%

As in previous investigations (Scott et al 1984) anionic detergent was used at 1.2% v/v diluted in sterile distilled water.

All disinfectants (used here and in 2.22 below) were freshly prepared and hypochlorite disinfectants were standardized by Iodometric titration (anon 1971).

2.22 Disinfectant test agents employed on test surfaces (Chapter 6)

Test agents used in Chapter 6 to assess disinfectant action on surfaces comprised Stericol (described above), a xylenol fluid solubilised with soap (brand name 'Clearsol' Tenneco Organics Ltd, Bristol), sodium hypochlorite solution (described above) and sodium dichloroisocyanurate (NADCC) containing 64.5% w/v available chlorine (brand name 'Clearon' Chlorchem Ltd, Widnes)

Solutions of these disinfectants were prepared in sterile distilled water to give the following use dilutions:

	Use-dilution
Stericol	2% v/v
Clearsol	1% v/v
Sodium hypochlorite	2,500 ppm
NADCC	2.500 ppm

2.23 Neutralization medium employed for cloths (Chapter 5)

Following experiments on the chemical disinfection of cloths, both disinfected and control cloths were transferred to a neutralizing medium prepared in quarter strength Ringer solution (described by Scott et al 1984) as follows:

3% Tween 80 (BDH Chemicals Ltd, Dagenham)

0.3% Lecithin

0.1% L. histidine (Sigma, Poole)

0.5% Sodium thiosulphate (May and Baker, Dagenham)

2.24 Neutralization medium employed for cloths and surfaces in the quaternary ammonium cloth trials (Chapter 9)

Using details of an investigation published by Babb et al (1981), contact plates containing nutrient agar and a 0.75% lecithin-Tween mixture (prepared by mixing 50g Tween 80 and 5g lecithin) were used for recovery of organisms from surfaces wiped with a quaternary ammonium cloth and from the cloth itself.

CHAPTER 3
REVIEW OF SAMPLING METHODS

Many different methods are available for sampling inanimate surfaces. Traditionally, techniques such as swabbing, rinsing and contact impressions with agar have been employed for sampling surfaces in the food and dairy industry, in factories and in hospitals. More recently rapid methods have been developed including the use of direct epifluorescent microscopy (DEM) and direct epifluorescent filter technique (DEFT) to assess microbial populations on food contact surfaces (Holah et al 1988).

Throughout this study, Rodac or agar contact impression plates were used for quantitative sampling of inanimate surfaces both in laboratory experiments and in field studies. The rationale for the use of contact plates in this way is based upon the following. Much of the work described in this thesis is concerned with estimating the transfer of bacterial contamination from one contaminated surface to a potentially more crucial surface rather than assessment of survival on surfaces per se. In practice, such transfer may be effected by several means but probably the primary vehicles as studied in this work are hand, cloths and other inanimate objects. Since the contact time between hands and cloths etc and a contaminated surface may be relatively brief, it was considered that the use of agar contact plates more closely mimics the practical situation than other sampling techniques commonly available such as swabbing and rinsing.

The use of agar contact plates for bacterial sampling of relatively clean contact surfaces has gained wide acceptance since its development by Walter and Hucker (1941). The further refinement of this method by Hall and Hartnell (1964) together with its current commercial availability and portability which facilitates the rapid collection of large numbers of surface samples have made it a popular technique. In previous field studies (Scott 1981 and Scott et al 1984) contact plates were used for the successful differentiation of 'hygiene levels' at environmental sites whilst facilitating handling of large numbers of samples in a field study.

The method is used to provide mainly quantitative information in the form of total counts per sample area. One of the problems of using contact plates rather than swab or rinse techniques is that they cannot be used to estimate counts where surfaces are heavily contaminated. For the purposes of this study, 250 colonies were considered to be the maximum count which could be determined accurately. This factor was not considered to represent a problem in this work which was mainly concerned with identification of the occurrence of heavy contamination on surfaces (ie, greater than 100 colonies/sample area) rather than differentiating between "levels of heavy contamination".

Problems associated with the use of hand held contact plates during sampling have been identified by

Brewer and Turner (1973); these include the difficulties in achieving consistent pressure when sampling and the problems of horizontal slippage resulting in smearing of colonies. For the studies described in this thesis, these problems were largely overcome by the use of contact plates which had been air-dried for one hour in a laminar flow cabinet to reduce horizontal slippage. For laboratory studies, contact plates were also weighted with a 200 grm weight whilst placed in contact with a surface for a timed 30 second period.

The relative efficiency of the various sampling techniques in recovery of organisms from surfaces has been investigated by a number of workers. Increased recovery of organisms from surfaces using swabs as opposed to contact plates was reported by Favero et al (1968) and Gilbert (1970). These workers suggested that mechanical break-up of clumps during swabbing gives rise to more colony-forming units. Angelotti et al (1961) described the agar contact plate as the method of choice for most quantitative bacterial sampling but concluded that the method was less efficient in removal of bacteria (especially from soiled surfaces) than some other sampling methods. A comparison of the contact plate method with swabbing techniques for enumeration of bacteriological contamination at 9 environmental sites in 64 homes was reported by Scott et al (1984). Contamination levels of

100 or more organisms/21-25 cm^2 were demonstrated more frequently using swab methods, but for some sites where low number of organisms were present, higher recovery rates were obtained using contact plates. When contamination levels from contact plate and swab techniques were compared according to rank order a good correlation was obtained. In a review of laboratory techniques used in test methods for surface disinfection procedures, Werner et al (1977) compared the use of swabbing and impression method. It was found that whereas the reliability of swabbing depended on the operator, the contact impression methods were more easily standardized and easy to perform. These workers claimed that using a single contact plate, regardless of the type of surface, only about 25% of the contamination was removed. This aspect is not of particular concern in this work which deals with transfer of contamination by a single contact between surfaces. Whyte et al (1989) reported on the development of mathematical models for calculating the efficiency of bacterial surface sampling techniques. It was found that when using pairs of Rodac plates for sequential sampling of laboratory benches, the sampling efficiency varied from 32% to 69% with a median efficiency of 47%

Throughout laboratory experiments reported in this work, there was found to be reasonable agreement between duplicate contact plate counts from within

a particular experiment, although in some cases, considerable differences were observed between experiments. This lack or reproducibility is a common factor associated with surface sampling (Pettit and Lowbury 1968, Werner et al 1977). Results are therefore used to identify situations where significant contamination may be encountered.

CHAPTER 4

LABORATORY STUDIES ON THE SURVIVAL OF CONTAMINATION ON CLOTHS AND WORKSURFACES AND ITS TRANSFER VIA CLOTHS, HANDS AND UTENSILS

4.1 INTRODUCTION

Previous studies as described in Chapter 1 pages 36 to 39 indicate that wet cloths and cleaning utensils, together with hand and food contact surfaces are important elements in cross-contamination.
In practice, other inanimate objects such as items of medical or patient care equipment or cooking equipment as well as the hands have been recognized as vehicles of contamination transfer. Reybrouck (1983) commented on the fact that in hospitals, inanimate objects such as instruments, thermometers, breathing apparatus and linen etc. can all serve as the vehicle of transmission. As discussed in Chapter 1, page 32, experts in food hygiene believe that the importance of cross-contamination as a contributory factor in food poisoning transfer is underestimated.
Gilbert (1986) indicated the need for separate worksurfaces and kitchen equipment for raw and cooked foods together with other measures in order to keep crosscontamination to a minimum.
Other workers have studied the role of hands in transferring contamination in hospitals but in such studies, it is a patient and not an inanimate surface that is usually considered as the source of contamination. There is little evidence available to suggest the extent to which contamination transferred by hand may have arisen from the environment rather than from a patient or staff. In a study carried out in 1977, Casewell and Philips identified nursing procedures which involved only brief slight contact with the patient's skin and yet resulted in the

De Wit *et al* (1979) reported experiments comparing the survival of *E.coli* and *Salmonella* on test surfaces. Results showed that whereas *Salmonella* was isolated for up to 6h from dry surfaces, *E. coli* could not be recovered beyond 4h and the authors concluded that *Salmonella* can be more resistant than *E. coli* to some external factors.

Studies of clinical isolates of *Acinetobacter calcoaceticus* on formica surfaces showed that certain strains were recoverable in low viable numbers up to 60h after inoculation (Musa *et al* 1990).

Field studies on the survival and recovery of contamination from worksurfaces have tended to concentrate on food preparation surfaces in the home and in catering establishments.

In reviewing the relationship between the survival of surface contamination and the transmission of disease, Sanborn (1963) reported that on one occasion *S.typhimurium* was isolated from a meat cutting board 12 days after the board was contaminated by turkey meat. In a survey of some 5,000 sampling positions in 100 catering establishments, Mendes *et al* (1978) found that 59% of bacteria isolated from surfaces were of faecal origin (coliforms), including *Salmonella* species isolated from preparation surfaces and hand contact sites. They also reported that *E. coli* survived less well on surfaces than other coliforms and suggested that hygiene surveys should measure total coliforms rather than relying upon the isolation of *E. coli* alone as an indicator of poor hygiene conditions.

transfer of 100-1000 viable organisms to the nurses' hands.

A number of laboratory studies have been carried out to determine survival and recovery of different species of bacteria from test surfaces which have been used to represent a variety of hard surfaces such as worksurfaces, floors, walls etc.

Lidwell and Lowbury (1950) and Lowbury and Fox (1953) carried out experiments which showed that Gram positive cocci can survive for long periods under dry conditions whereas Gram negative bacilli die rapidly. Lowbury and Fox (1953) also found that the presence of serum afforded some protection against drying. Ayliffe et al (1967) studied the survival and recovery of Staph. aureus from vinyl surfaces over a period of 7 days and reported that the numbers recovered declined steadily over the test period. Even so, 46% of the organisms were still recoverable âfter one day and 22% after two days. In an experiment investigating the survival of wound pathogens under different environmental conditions, Pettit and Lowbury (1968) demonstrated that a smaller proportion of Gram negative bacilli than Gram positive cocci survived after drying under clean conditions. Further, it was shown that the numbers of survivors rapidly decreased during the initial 30-90 min. period of drying, after which there was little or no further death and the pattern of survival was then similar for both Gram positive and Gram negative test organisms.

In a Dutch study of homes where a case of salmonellosis had occurred in an infant, it was found that kitchen sinks and worksurfaces were often contaminated with the same serotypes resulting in cyclic infections and indicating significant survival of these organisms (Van Schothorst et al 1978).

From a sample of 21 domestic food preparation worksurfaces Finch et al (1978) did not isolate any Gram negative bacilli. In a larger survey of 1163 domestic food preparation surfaces reported by the author (Scott 1981) it was found that although micrococci and Bacillus spp. predominated, the following organisms were also isolated and identified (percentage frequency of occurrence shown in brackets): E. coli (5.6%) K.pneumoniae (5.3%), C. freundii (8.1%), Ent.cloacae (5.5%), Ps.aeruginosa (1%), Ps.maltophilia (9.1%) and other Pseudomonas spp (23.4%). High colony counts of 100 or more/25cm^2 were recorded for 23.3% of worksurfaces. The likelihood of massive contamination of wet cloths and other cleaning utensils and the potential for spread of such contamination has been recognized by many workers.

Ayliffe et al (1967), Litsky and Litsky (1968) and Westwood et al (1971) reported on the potential for contamination spread as a result of mopping techniques in hospitals. Whitby and Rampling (1972) and Baird et al (1976) found that wet cloths and cleaning utensils in hospitals were frequently contaminated with Ps.aeruginosa.

In a survey of domestic dishcloths, Davis et al (1968) reported total counts of up to 1 x 10^8/cloth and on occasions found E.coli present in numbers as high as 10^7/cloth. Finch et al (1978) and Scott et al (1982) in surveys of domestic dishcloths reported on high counts and the presence of contamination of enteric origin.

Tebbutt (1986) found that 74% of cloths used for wiping food shop surfaces were contaminated with one or more of the following: E.coli, Staph.aureus, Strep.faecalis and Clostridium perfringens. E.coli was isolated from 56% of cloths, nearly half containing more than 10^5 colonies. Davis et al (1968), Gilbert (1969) and Tebbutt (1986) showed that wiping hard surfaces with contaminated cloths may result in contamination of hands, equipment and other surfaces.

Whilst the potential for transfer of contamination from and via cloths and worksurfaces is well recognised, a survey of the literature indicates that few workers have made a study of this aspect in the laboratory. During the course of an investigation of self-disinfecting cloths, Babb et al (1981) set up model worksurfaces which were artificially contaminated and reported the transfer of E. coli, Ps aeruginosa and Staph. aureus from one surface to another via non disinfected cloths.

A skin contact transfer model established by Marples and Towers (1979) was adapted to measure the ability of a range of organisms representative of bacterial species mostly responsible for H.A.I. to transfer from contaminated fabrics to hands and from hands to sterile

fabrics (Mackintosh and Hoffman 1984). Staph. saprophyticus (representing Staph. aureus) transferred well to the hands but less well from the hands to another fabric. Pseudomonas aeruginosa, K. aerogenes and Serratia marcescens transferred reasonably well both from fabric to hands and hands to fabric whereas E. coli and Strep. pyogenes did not. In common with the findings of other workers (Mendes et al 1978 and De Wit et al 1979), E. coli was found not to be typical of the Gram negative bacilli in its ability to survive on skin and in the environment.

De Wit et al (1979) performed an experiment to determine the extent to which the preparation of frozen chickens contaminated with an indicator organism (E. coli K12) caused cross-contamination. It was reported that 74% of dishcloths together with a large number of other kitchen objects became contaminated with the indicator organism.

Despite these investigations, little attempt has been made to quantify transfer risks from contaminated surfaces. In this chapter, laboratory experiments are described which determine the extent to which survival of organisms on cloths and laminate worksurfaces may be associated with cross-contamination via the hands, cloths and stainless steel surfaces.

4.2 Survival of contamination on clean and soiled worksurfaces and cloths

The first section of this Chapter describes experiments carried out in the laboratory to determine the survival of laboratory (ls) and wild type (wt) strains of test organisms on both clean and soiled laminate squares representing worksurfaces and on clean and soiled cloths.

4.2.1 Method

4.2.1.1 Inoculation and Sampling of Laminate Squares

Bacterial test suspensions (100 ul) in either broth or water (Chapter 2 page 61) to simulate soiled and clean conditions respectively were pipetted onto laminate squares (Chapter 2 page 63) to give a total inoculum size of approximately 300 organisms. Laminate squares were stored in assay dishes at 30°C and 40-45% RH and at this temperature and humidity the drops dried in 90 mins.

Laminate squares were sampled using tryptone soya agar contact plates (as described in Chapter 2 page 65). Sampling was attempted at time 0 (ie immediately after inoculation of test suspension onto the laminate square) and at 1h, 4h and 24h after inoculation. Two contact plate impressions were taken from two separate drops on one test worksurface at each recovery time. The experiment was carried out twice. Each result represents the average count from two contact plates (counts/25cm^2)

4.2.1.2 Inoculation and sampling of cloths

Using a 5ml Finn pipette, 3 mls of diluted test suspension in water was added to sterile clean and soiled cloth portions (Chapter 2 page 64) folded in Petri dishes giving a total inoculum per cloth portion (1260 cm^2)

of approximately 1.5×10^5 or 120 organisms per cm^2. Organisms inoculated onto cloths were recovered onto tryptone soya agar contact plates. Sampling by contact plate was attempted at the following times: time 0 (immediately following inoculation), 1h, 4h, 6h, 24h and 48h after inoculation. Throughout this period, cloths were stored in closed Petri dishes at room temperature (18-20°C) and 60% RH. After 48h storage in this manner, the cloths were found to be dry to the touch.

Two contact plate impressions were taken from a cloth at each sampling time. The experiment was carried out twice. Each result represents the average count from two contact plates.

4.2.2 Results

Although there was reasonable agreement between duplicate contact plate counts for a particular experiment, in some cases considerable differences were observed between experiments. This lack of reproducibility is a common feature associated with surface sampling as discussed in Chapter 3. Results were therefore used to identify situations where significant contamination may be encountered.

4.2.2.1 Survival on worksurfaces

The average number of organisms recovered from both soiled and clean worksurfaces are shown in Table 8. Results indicate that a substantial proportion of the inoculum was recovered at 0 and 1h from both clean and soiled surfaces during which time surfaces remained damp. Some species showed an initial increase in numbers

Table 8. Survival of organisms on clean and soiled laminate surfaces

	Number of colony-forming units recovered per 25 cm^2 contact plate																	
	Escherichia coli				Klebsiella aerogenes+		Klebsiella pneumoniae*		Pseudomonas aeruginosa+		Salmonella abony+		Salmonella spp*		Staphylococcus aureus			
	+		*												+		*	
	Soiled conditions																	
Total surface inoculum cfu/100 ul	250	200	250	250	300	120	370	-	200	170	400	350	300	-	320	550	204	500
Recovery times (h)																		
0	153	49	193	85	52	37	225	-	164	67	167	17	190	-	125	149	116	115
1	200	7	248	79	85	40	268	-	156	36	162	14	199	-	147	118	157	146
4	0	4	20	2	4	3	35	-	2	12	20	3	37	-	111	80	37	160
24	<1	2	3	0	0	0	0	-	1	5	0	3	13	-	42	27	78	7
	Clean conditions																	
Total surface inoculum cfu/100 ul	300	260	290	100	437	110	150	-	150	140	300	400	375	-	190	100	200	500
Recovery times (h)																		
0	240	84	181	49	52	41	44	-	108	44	112	18	125	-	83	24	141	176
1	157	38	172	47	61	22	4	-	94	24	103	17	131	-	75	12	193	102
4	0	0	0	3	0	1	1	-	3	0	0	0	1	-	2	3	7	2
24	0	0	1	0	0	0	3	-	1	0	0	0	1	-	0	1	5	0

+Laboratory strains: * wild-type strains

between 0 and 1h suggesting multiplication on surfaces. For clean surfaces, lethal drying effects were clearly apparent by 4h with little or no survival at this time and subsequently at 24h. Under soiled conditions, E. coli (wt), Kl. pneumoniae (wt), Salm. abony (ls) and Salmonella spp (wt) and Staph. aureus (ls and wt) all showed indications of survival in significant numbers of 20 or more cfu's/contact plate for up to 4h. Staph aureus (ls and wt) survived in significant numbers for up to 24h on soiled worksurfaces.

4.2.2.2 Survival on cloths

The average number of organisms recovered from both soiled and clean cloths in each of two experiments over 48h are shown in Table 9.

Results indicate that although numbers of organisms on clean cloths declined over the drying period, with the exception of E. coli (ls), Kl. pneumoniae (wt) and Staph. aureus (wt), recovery at 4h was greater than 20 cfu/25cm^2. At 24h, the majority of clean cloths contained less than 20 cfu/25cm^2 with the exception of Staph. aureus (ls) but for Kl. aerogenes (ls) and Ps. aeruginosa (ls) there was regrowth of residual survivors at 48h. Soiled cloths showed generally higher levels of survival and recovery onto contact plates with only Staph. aureus (ls and wt), Salmonella spp (ls and wt) and E. coli (ls) reduced to less than 20 cfu/25cm^2 at 24h and 48h.

Although, with the exception of Ps. aeruginosa (ls), there was an initial reduction at 4h, substantial regrowth of residual survivors occurred with 24h, maintained at 48h.

Table 9. Survival of organisms on clean and soiled cloths

	Number of colony-forming units recovered per 25 cm^2 contact plate																	
	Escherichia coli +		Escherichia coli *		Klebsiella aerogenes+		Klebsiella pneumoniae*		Pseudomonas aeruginosa+		Salmonella abony +		Salmonella spp.*		Staph. aureus +		Staph. aureus *	
Soiled conditions																		
Inoculum per 25 cm^2 of cloth	2225		7075		2675		8750		3125		4350		7800		36662		5823	
Recovery times (h)																		
0	217	T	T	T	T	T	T	180	T	T	180	T	T	T	T	T	T	T
1	77	41	T	T	T	T	165	45	T	T	50	T	100	135	T	T	154	60
4	50	37	175	210	161	241	73	9	T	T	33	100	52	71	T	91	2	23
24	0	0	T	68	22	T	T	T	T	T	7	15	4	7	35	6	5	8
48	0	0	T	45	T	T	T	T	T	T	3	3	1	0	5	0	0	0
Clean conditions																		
Inoculum per 25 cm^2 of cloth	2375		5750		2825		1750		4912		3937		7600		22500		4325	
Recovery times (h)																		
0	146	174	T	T	100	124	250	245	226	161	143	170	T	T	T	T	T	T
1	121	105	T	T	28	51	12	20	77	47	54	46	T	T	T	T	46	92
4	8	21	231	225	36	29	1	1	39	23	30	43	204	134	T	472	12	11
24	0	2	20	3	1	9	0	0	T	T	14	23	27	12	135	45	1	1
48	0	0	18	3	T	T	0	0	T	T	5	8	15	2	9	7	0	0

+Laboratory strains; * wild type strains; T, too numerous to count

4.3 Survival of contamination on clean and soiled worksurfaces maintained in a moist condition

Results from the previous section for laminate squares represent recovery of contamination from squares stored at 30°C for 24h. Under these conditions, inocula dried in 90 mins so that subsequent sampling times (ie 4h and 24h) involved recovery from dry laminate squares.

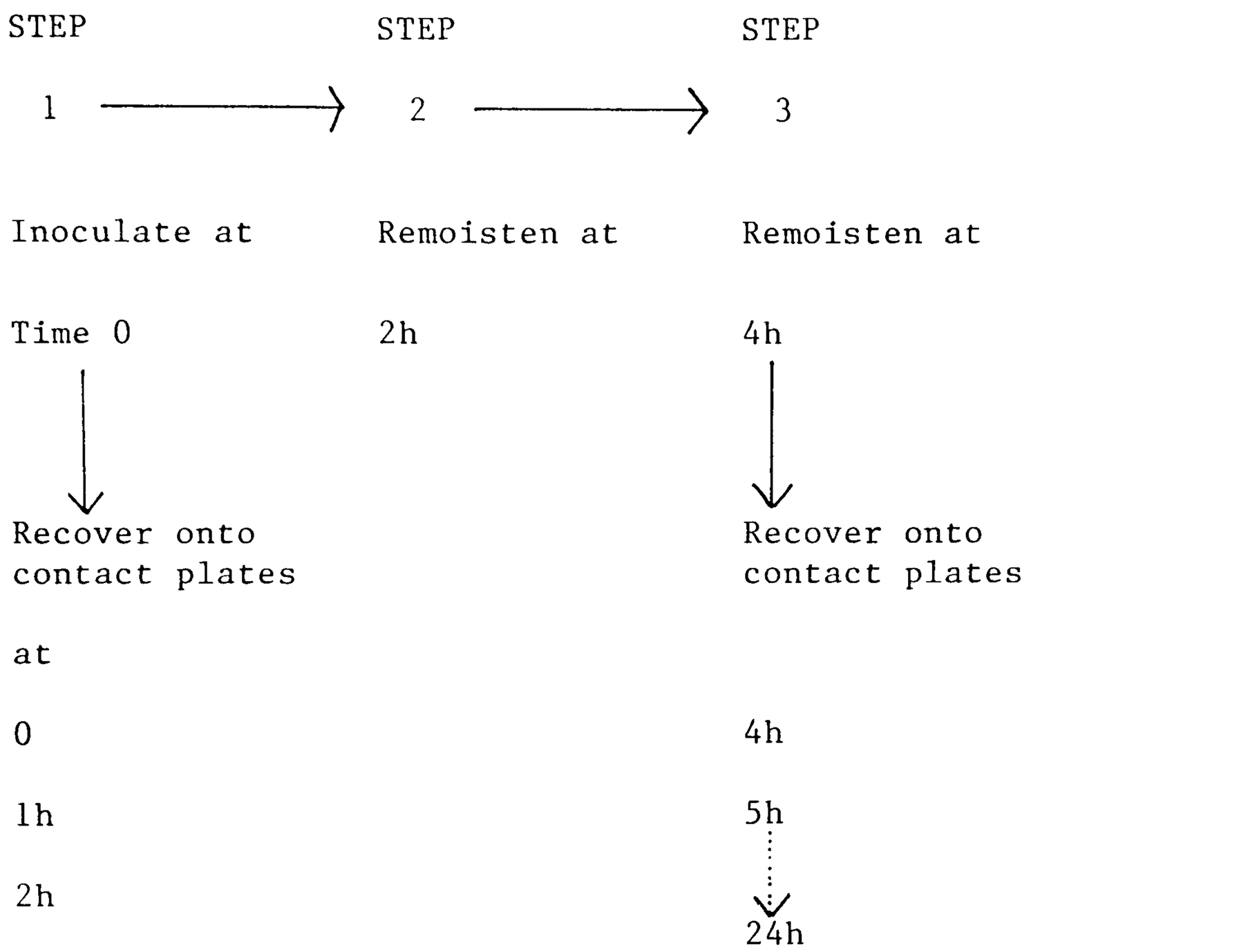
The following experiment was carried out to determine the extent to which recovery of contamination might be increased by maintaining the laminate squares under moist conditions. Test organisms chosen for this experiment were those which showed significant survival on dry laminate squares (Table 8).

4.3.1 Method

Bacterial test suspensions (100 ul) in either broth or water were pipetted onto laminate squares to give a total inoculum of approximately 300 organisms. Laminate squares were stored in assay dishes at 30°C and 40-45% RH. Laminate squares were sampled using tryptone soya agar contact plates. Recovery times are summarized in Fig.3. Recovery from 2 separate drops of inocula using 2 contact plates was attempted at time 0 (immediately after inoculation) and at 1h and 2h. After 2h and 4h, the remaining drops were remoistened by the addition of 100ml sterile distilled water. Recovery was attempted after remoistening at 4h, 5h and 24h.

The whole experiment was performed twice for Staph. aureus and three times for E. coli and Salmonella. The results represent an average count from 4 contact plates at each recovery time for Staph. aureus and for E. coli and Salmonella the results represent an average count from 6 contact plates.

Fig 3. Summary of method for the recovery of test organisms from laminate squares maintained in a moist condition



4.3.2 Results

Results comparing recovery of test organisms from laminate squares maintained in a moist condition and sampled over 24h are shown in Table 10 . Results for recovery from dry laminate squares as determined in Section 4.2.2.1. are also presented for comparison.

Under soiled conditions, remoistening at 2h and 4h resulted in an increase in recovery at 5h for E. coli and Staph. aureus wild types but not Salmonella. Comparing the recovery at 4h with that from dry surfaces, it was found that maintaining the soiled surfaces in a moist condition produced much higher recovery of E. coli (wt) (>200 cfu's/contact plate as compared with 20 cfu's/contact plate) and Staph. aureus (wt) (141 cfu's/contact plate as compared with 37 cfu's/contact plate) but not for Salmonella (28 cfu's/contact plate from a moist surface as compared with 37 cfu's/contact plate from a dry surface).

Under clean conditions, the presence of moisture produced little increase in recovery for E. coli and Salmonella at 4h. By contrast, maintaining the clean surfaces in a moist condition produced much higher recovery of Staph. aureus (wt) (75 cfu's/contact plate from a moist surface as compared with 7 cfu's/contact plate from a dry surface).

Recovery of E. coli and Salmonella wild types at 24h was negligible regardless of conditions (moist or dry, clean or soiled) and maintaining the surfaces in a moist condition did not appear to influence the recovery at 24h of Staph. aureus.

Table 10. The number of C.F.U's of test organisms recovered onto contact plates from laminate squares maintained in a moist condition over 5h

	SOILED CONDITIONS					
	MOIST LAMINATE SQUARES			DRY LAMINATE SQUARES		
	TEST ORGANISMS					
	E. coli Wild type	Salmonella Wild type	Staph. aureus Wild type	E.coli Wild type	Salmonella Wild type	Staph. aureus Wild type
Inoc. size 100/ul	307	271	220	250	300	204
Recovery times (h)						
0	137	126	117	193	190	116
1h	158	178	144	248	199	157
2h	136	91	169	-	-	-
Remoistening 4h	>200	28	141	20	37	37
5h	>200	6	163	-	-	-
24h	0	<1	67	3	13	78

Table 10 (Cont) The number of C.F.U's of test organisms recovered onto contact plates from laminate maintained in a moist condition over 5h

	CLEAN CONDITIONS					
	MOIST LAMINATE SQUARES			DRY LAMINATE SQUARES		
	TEST ORGANISMS					
	E. coli Wild type	Salmonella Wild type	Staph. aureus Wild type	E. coli Wild type	Salmonella Wild type	Staph. aureus Wild type
Inoc. size 100/ul	265	280	177	290	375	200
Recovery times (h)						
0	114	120	129	181	125	141
1h	131	116	191	172	131	193
2h	20	41	136	-	-	-
Remoistening 4h	2	0	75	0	1	7
5h	<1	0	79	-	-	-
24h	0	<1	<1	1	1	3

4.4 Survival of contamination on soiled cloths maintained in a moist condition

Results from the previous section on cloths (page 84) indicate that large numbers of organisms were still recoverable at 48h from dry cloths. In practice, it is likely that cloths would be in use again within a 48h period and in a situation in which the cloths had not been subjected to disinfection then any contamination on the cloths would then be subject to remoistening. The following method was devised to investigate the effect of remoistening contaminated cloths after 48h storage.

4.4.1 Method

Following sampling at 48h as described in section 4.2.1.2 page 81 , all the soiled cloths in that experiment were remoistened by the addition of 3ml sterile distilled water. Recovery onto tryptone soya agar contact plates was attempted at 1h, 4h and 24h after remoistening. During this time, the cloths were stored in closed dishes at room temperature. For each test organism the experiment was carried out twice. At each recovery time, 2 contact plate impressions were taken and the results are presented as the average count of 4 contact plates taken from 2 cloths.

4.4.2 Results

The results showing the effect of remoistening soiled cloths on the recovery of test organisms onto contact plates are shown in Table 11.

The results indicate that those organisms which were recovered in numbers 'too numerous to count' at 48h *E. coli* (wt), *K. pnemoniae* (wt), *K. aerogenes* (ls)

Table 11. The effect of remoistening soiled cloths on the recovery of test organisms onto contact plates

Test organisms	Recovery times	Dry cloth (48h after initial inoculation)	Cloth (1h after remoistening)	Cloth (4h after remoistening)	Cloth (24h after remoistening)
		C.F.U's per $25cm^2$ contact plate			
E.coli (wt)		TNTC	TNTC	TNTC	TNTC
E.coli (ls)		0	0	0	0
K.pneumoniae (wt)		TNTC	TNTC	TNTC	TNTC
K.aerogenes (ls)		TNTC	TNTC	TNTC	TNTC
Ps.aeruginosa (ls)		TNTC	TNTC	TNTC	TNTC
Salmonella (wt)		<1	0	0	--
S.abony (ls)		3	3	0	0
Staph.aureus (wt)		0	12	52	--
Staph.aureus (ls)		2	6	4	0

and Ps. aeruginosa (ls), remained 'too numerous to count' at 1h, 4h and 24h after remoistening. For those organisms (except Staph. aureus (wt)) which were recovered in either very low numbers or were not recoverable at 48h (E. coli ls, Salmonella wt, S. abony ls and Staph. aureus ls) remoistening the cloths did not result in any increase in the numbers recovered. However, for Staph. aureus (wt), although not recoverable at 48h there was some increase in recovery at 1h and 4h after remoistening (12 cfu's/25cm^2 and 52 cfu's/25cm^2 respectively).

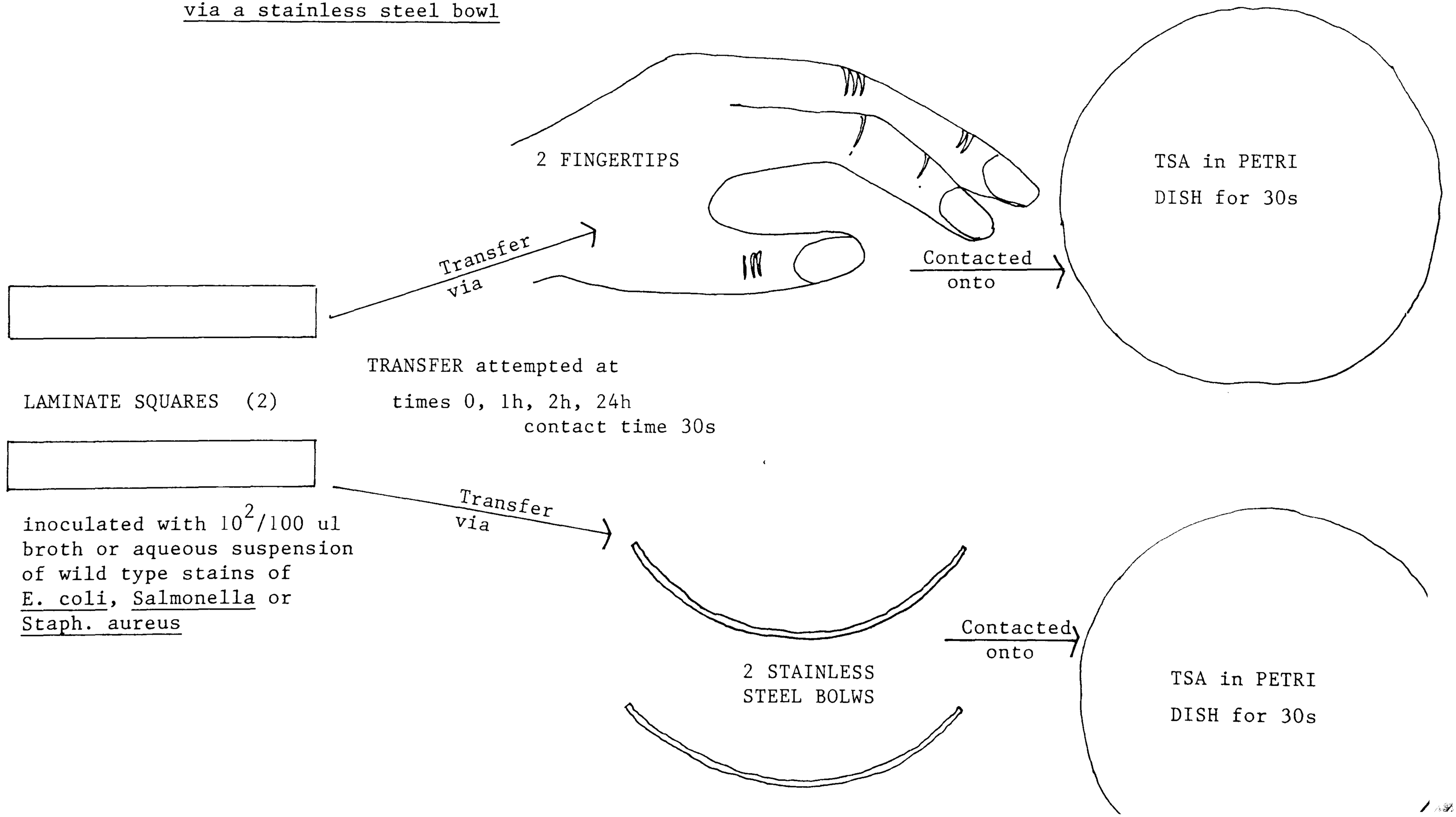
4.5 Transfer from soiled worksurfaces via the fingertips and a stainless steel bowl

The results described in the previous section clearly establish the potential for some species of bacteria to survive in significant numbers on soiled worksurfaces for a number of hours. The experiments to be described in this section were designed to investigate the potential for transferring organisms from worksurfaces via the fingers and via an inanimate object onto other surfaces. The inanimate object selected for this study was a small stainless steel bowl chosen to represent the kind of items used in kitchens, in the manufacturing environment and in hospital wards (the so-called'patient-care items'). The surface to which the organisms were transferred (in this case, agar in petri dishes), was intended to represent potentially more crucial surface, ie, surfaces to which transfer of contamination could pose a potential risk such as clean preparation areas, instrumentation and equipment, high risk foods or even a patient in hospital. Test organisms chosen for this study were those which showed significant survival on laminate surfaces for up to 4h (see Table 8).

4.5.1 Method

Using a method as outlined in Fig 4 transfer of organisms via the fingertips or via the steel bowls was attempted at time 0 (immediately after inoculation) and at 1h, 2h and 24h after inoculation. Throughout this period,

Fig 4. Transfer of organisms from worksurfaces to other surfaces via the fingers or via a stainless steel bowl



the worksurfaces were stored in open assay dishes at 30°C and 40-45% RH. Transfer was attempted using either two fingertips (middle and forefinger of the right hand) or a small stainless steel bowl. Fingertips and bowl were preswabbed with 70% alcohol which was allowed to evaporate before contact with the inoculated worksurface. At each transfer time, the fingertip or steel bowl was placed in contact with the whole of an inoculated drop on the worksurface for 30s. Contact was made with separate drops using 2 fingers or 2 bowls at each transfer time. The bowl was weighted down with a 200 grm weight. After 30s the 2 fingertips and 2 bowls were then contacted onto tryptone soya agar for a further 30s. The experiment was carried out twice for each test organism. Colony forming units on agar in petri dishes were counted and are presented as the average counts per fingertip or per bowl (from a total of 2 fingertips or 2 bowl counts) for each experiment.

4.5.2 Results

Contamination transferred from a soiled worksurface via the fingertips (total surface contact area approx. 2 cm^2) or a stainless steel bowl (surface contact area approx. 1 cm^2) is shown in Table 12.

The results indicate that for all 3 test organisms substantial numbers (between 50 and 100) can be transferred from a soiled worksurface for upto 1h via either the fingertips or a steel bowl.

Table 12 Transfer of organisms from a soiled laminate surface to the fingers or stainless steel bowl

	Number of colony-forming units recovered per 25 cm^2 contact plate											
	Fingertip						Stainless steel bowl					
	Escherichia coli*	Salmonella spp.*	Staphylococcus aureus*	Escherichia coli*	Salmonella spp.*	Staphlococcus aureus*	Escherichia coli*	Salmonella spp.*	Staphylococcus aureus*	Escherichia coli*	Salmonella spp.*	Staphlococcus aureus*
Total surface inoculum cfu/100 ul	330	200	270	560	210	210	330	200	270	560	210	210
Recovery times (h)												
0	59	55	42	59	57	49	59	53	49	73	38	46
1	69	62	55	78	99	85	56	49	54	95	46	40
2	25	80	<1	11	46	82	30	17	31	0	1	34
24	1	5	0	6	18	20	0	0	0	0	0	3

* Wild type strains

Even at 2h, by which time the drops of inocula had dried, significant numbers (20 or more cfu) could still be transferred via both fingertips and steel bowl, with the exception of Salmonella (wt) via the fingertips. At 24h, by which time few species would remain viable on the worksurface (see Table 8 page 83), only Staph. aureus (wt) was transferred via the fingertips in significant numbers (approximately 20 cfu).

4.6 Transfer from cloths to fingers and worksurfaces

It has long been established that cross-contamination may occur resulting from the use of cloths (as reviewed on page 79). Using soiled cloths this section describes experiments designed to investigate quantitatively the potential for transfer of contamination via cloths to fingers and to worksurfaces.

Test organisms chosen for this study were those which showed significant survival on laminate squares (Table 8) and which had been used by other works in experiments involving transfer from contaminated fabrics (Mackintosh and Hoffman 1984).

4.6.1 Method

Using a Finn pipette, 3ml volumes of test suspension in tryptone soya broth were added to sterile soiled cloth portions (Chapter 2 page 64) stored in petri dishes, to give a total inoculation of approximately 1.5×10^5. Transfer of organisms to the fingertips and to a worksurface was determined at time 0 (following inoculation) and 1, 4, 24 and 48h after inoculation. Prior to contact with the inoculated cloths, the 2 fingers (middle and forefinger) of the right hand were swabbed with 70% alcohol which was then allowed to evaporate. The fingertips were then placed firmly in contact with cloths for 30s. The fingertips were then further contacted onto tryptone soya agar for 30s. Finally, using sterile disposable gloves, the cloth portion was used to wipe the entire surface of a sterile laminate square. The laminate square was then sampled with 2 tryptone soya agar contact plates.

The experiment was carried out twice. The results are presented as the average of two counts per fingertip or per worksurface for each experiment.

4.6.2 Results

Contamination transferred to fingertips or a worksurface from contaminated soiled cloths is shown in Table 13 .

The results indicate that apart from E. coli (wt) transferred to fingertips and Staph. aureus (wt) transferred to a worksurface significant numbers (20 or more cfu) of all three species were transferred over the first 4h after inoculation. Where the numbers of organisms increased on the cloths over 4 h due to regrowth of residual survivors (as indicated in Table 9 page 85) this was accompanied by increased transfer.

Table 13 Transfer of organisms from a soiled cloth to the fingers or a work surface

	Number of colony-forming units recovered per 25 cm^2 contact plate					
	Fingertip			Laminate surface		
	Escherichia coli*	Klebsiella aerogenes†	Staphylococcus aureus*	Escherichia coli*	Klebsiella aerogenes†	Staphylococcus aureus*
Total inoculum per 25 cm^2 cloth	976	714	2976	976	714	2976
Recovery times (h)						
0	5 8	48 92	101 113	31 37	41 43	35 31
1	5 7	60 34	77 153	22 31	34 39	28 27
4	4 3	41 24	91 57	20 22	22 24	8 8
24	T T	T T	T T	T T	T T	T T
48	T T	T T	T T	T T	T T	T T

† Laboratory strains; * wild type strains; T, too numerous to count

4.7 Recovery from cloths contaminated by contact with chicken

Previous experiments in this chapter investigated the survival and recovery of test organisms inoculated onto clean and soiled cloths (sections 4.2 and 4.4). In order to determine whether potentially pathogenic organisms and Gram negative species in particular, could be recovered from naturally contaminated cloths, the following method was devised.

4.7.1 Method

Five sterile clean cloths (Chapter 2.16) were wiped over the surface of a fresh chicken portion and were then returned to glass petri dishes for storage at room temperature for 21 days. Recovery onto MacConkey agar contact plates was attempted on days 1, 3, 7 and 21 after the cloths had been contaminated by wiping the chicken surface. Colonies grown up on the contact plates were steaked onto MacConkey agar in Petri dishes and following incubation, identification of individual species was performed using colonial characteristics, Gram staining and A.P.I 20E, as described in Chapter 2 page 60.

4.7.2 Results

The results for recovery of Gram negative organisms from five clean cloths contaminated by contact with chicken are shown in Table 14.

For all 5 test cloths, confluent growth on contact plates was observed at all recovery times over 21 days.

E. coli was recovered from all 5 cloths for up to

Table 14 Recovery of Gram negative organisms onto contact plates from clean naturally contaminated cloths over 21 days

Recovery time (days)	C.F.U's/25cm^2 contact plate and isolated species				
	Cloth sample				
	1	2	3	4	5
1	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coil*
3	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coli* *Citrobacter* spp	TNTC *E. coli*
7	TNTC *E. coli*	TNTC *E. coli*	TNTC *E. coli*	--	TNTC *E. coli*
21	TNTC *Aeromonas hydrophila*	TNTC *Proteus vulgaris*	TNTC *Aeromonas hydrophila*	TNTC Unidentified oxidase +ve 'Gram negs.'	TNTC *Aeromonas hydrophila* & *E. coli*

TNTC - Too numerous to count (confluent growth)

7 days and was the predominant organism recovered over this time. At 21 days E. coli was recovered from 1 of the 5 cloths whilst other organisms recovered at this time included Aeromonas hydrophila and Proteus vulgaris.

4.8 Discussion

Results from the first part of this chapter indicate the extent of bacterial survival and growth on laminate surfaces and on cloths. Drying, as expected, produced substantial reductions in recoverable organisms and, for clean worksurfaces, achieved satisfactory decontamination. Both Gram positive and some Gram negative species were recoverable in significant numbers from soiled surfaces and from clean and soiled cloths for up to 4h and in some cases up to 24 and 48h. For cloths certain organisms showed initial decline in numbers followed by a subsequent increase indicating adaption of organisms enabling multiplication on relatively dry cloths.

The results suggest that bacterial survival and regrowth on surfaces depends on a number of factors and is largely unpredicatable. Factors which have been found to have an effect upon the survival of bacteria attached to dry solid surfaces include temperature, relative humidity and species (Sleesman and Leben 1976 and McEldowney and Fletcher 1988). Recovery from cloths was generally higher than from surfaces (except for Staph. aureus). This probably relates to the higher inoculum size used and the slow drying rate of the cloths. As reported by Lowbury and Fox (1953) and Rathmachers and Borneff (1977) soiling is another important factor in preserving viability on hard surfaces. For cloths, soiling also encouraged regrowth of residual survivors although for Kl. aerogenes (ls) and Ps. aeruginosa (ls) regrowth occurred with clean

as well as soiled cloths. Although as found by Pettit and Lowbury (1968) and Rathmachers & Borneff (1977), survival of Gram positive species on laminate work surfaces was greater than that of Gram negative species, this was not the case with cloths. This may be due to differences in drying rate and nutrient availability between hard and cloth surfaces; Rathmachers and Borneff (1977) suggest that survival of Staph. aureus in moist situations under conditions of nutrient limitation may be less than that of Gram negative species. The action of remoistening surfaces and cloths produced varied results for the different test organisms and suggests that moisture is not the only factor in determining the survival and recovery of organisms from hard surfaces and from cloths. When Gram negative species were compared, it was found that E. coli (ls) was particularly sensitive to drying on both cloths and laminate surfaces, but survival of other Gram negative organisms varied according to the nature of the surface and the presence or absence of soil; De Wit et al (1979), found E. coli to be more sensitive than Salmonella to drying on test surfaces. Mendes et al (1978) reported that E. coli survived less well on kitchen surfaces than other coliforms. Some differences between laboratory and wild strains were observed, but there was no pattern which might suggest that wild strains should be used in preference to laboratory strains for assessing surface hygiene in laboratory experiments.

In the later sections of this chapter (Sections 4.5 and 4.6) contamination transfer by the fingers, cloths or a stainless steel surface was studied. Results suggest that, where contaminated surfaces come into even relatively brief contact with the fingers or an inanimate surface, significant numbers of organisms can be transferred which are recoverable onto an agar surface. Although transfer was reduced following storage of laminate surfaces, surface survival up to 2 h for all three species and up to 24 h for Staph. aureus was sufficient to allow significant transfer by the fingers or a stainless steel bowl. Similarly, where contaminated cloths were handled or applied to a clean laminate surface, significant transfer occurred. Transfer from cloths was generally greater than from laminate surfaces, giving contamination levels 'too numerous to count' at 24 and 48h. This correlates with higher contamination levels occurring in stored cloths. For transfer experiments (Table 13) soiled cloths were inoculated with broth rather than aqueous suspensions of test organisms as used for survival studies (Table 9) The results showed that the additional soiling facilitated the regrowth of E. coli, Staph. aureus and Klebsiella aerogenes.

In assessing hazards of cross contamination, it must be borne in mind that contamination applied to cloths and surfaces in these investigations was relatively low compared with levels which have been reported under in use conditions. Investigations suggest that in use contamination of cloths may

range from 10^3 to 10^8 organisms/cm^2 (Davis et al 1968, Mendes et al 1978, Scott et al 1982), whilst Gilbert & Maurer (1968) and Gilbert (1970) suggest that surface contamination following contact with food may be of the order 10^2 - 10^6 organisms/cm^2. Clinical investigations indicate that infection risks depend on numbers of organisms transferred. McCullough & Eisele (1951) reported that the infective dose of salmonellas may be 10^6 organisms or much lower. Outbreaks involving chocolate and Cheddar cheese suggests that the infective dose may be as little as 50-100, and less than 10 organisms respectively (Gill et al 1983; Greenwood & Hooper 1983; D'Aoust 1985). For toxin-producing species such as Bacillus cereus, Clostridium perfringens and Staph. aureus, it is accepted that the infective dose is greater than 10^6/g food, but transfer of even small numbers of these organisms to food increases the risk of multiplication to hazardous levels under conditions of poor storage etc.

From laboratory experiments described here,it is concluded that where contaminated surfaces or cloths containing even relatively low numbers of organisms come into contact with the fingers and other surfaces (eg a stainless steel bowl or a clean laminate surface), organisms may be transferred in sufficient numbers to represent a potential infection hazard. Although drying plays an important part in maintenance of hygiene in the kitchen and other environments, drying per se cannot be relied upon to prevent transfer of

contamination from laminate and cloth surfaces involved in potentially hazardous situations. The investigation emphasizes the importance of good hand hygiene and adequate decontamination procedures applied to cloths, laminate surfaces, utensils and other food contact surfaces during handling and/or preparation of food and in other critical environments.

CHAPTER 5

LABORATORY STUDIES ON THE DISINFECTION OF CLOTHS

5.1 INTRODUCTION

The results described in the previous chapter establish that bacterial contamination can survive in cloths and is recoverable by brief contact over substantial periods of time. Further experiments confirm the potential for cross-contamination involving cloths as the vehicle of transfer when contamination from cloths was deposited on fingertips and worksurfaces and transferred onto a further contact surface. These findings indicate the need for effective procedures for the decontamination of re-usable cloths but to date only limited data has been published. Westwood et al (1971) found that laundering and adequate drying provided effective decontamination of heavily contaminated mops whereas a "good phenolic disinfectant" at high concentrations was ineffective. However, even following effective decontamination the build up of contamination was found to be rapid upon re-use and daily decontamination was recommended. Walter and Schillinger (1975) also reported on studies showing that laundering at 54°C (8-10 mins) followed by adequate drying achieved acceptable decontamination for domestic linens. A 10-13 min wash-cycle at 60°C plus the addition of sodium hypochlorite was recommended for heavily contaminated linen from health care facilities. It was also found that the Gram positive Staph. aureus is more resistant to washing and drying than the Gram negative K. pneumoniae.

In an evaluation of domestic disinfectants under in-use conditions, Scott et al (1984) reported that although effective decontamination of dishcloths and cleaning cloths may be achieved by use of disinfectants, benefits were short-lived.

Acceptable reductions in contamination levels were achieved immediately following the use of phenolic and sodium hypochlorite disinfectants but counts began to rise again within 3h of disinfection, even though the cloths were not re-used during this period. Detergent and hot-water cleaning of cloths actually resulted in an initial increase in colony counts from cloths.

An innovation in cloth disinfection was examined by Babb et al (1981). Cloths which were impregnated with a blend of cationic disinfectants so as to be 'self-disinfecting' were tested. Laboratory studies indicated that the impregnated cloths were rapidly self-disinfecting and would not readily transfer bacteria from one surface to another over the first day's use although activity declined on subsequent days. In practice, the cloths were found to be neutralized rapidly by continued immersion in water or following contact with detergent and were therefore considered not to be reliable.

In this chapter, washing, drying and disinfection procedures for decontamination of cloths contaminated by use in kitchen environments are investigated.

5.2 The effect of a combination of detergent wash followed by low temperature drying on contaminated cloths

Results from a previous experiment (Chapter 4 page 84) indicate that under soiled conditions slow drying at room temperature cannot be relied upon to successfully decontaminate cloths. It is commonly recommended that contaminated cloths should be "washed and dried" and the following method was devised to investigate the efficiency of this treatment.

5.2.1 Method

On return to the laboratory, naturally contaminated cloths (Chapter 2, page 64) were aseptically divided into 4 portions (of approximately 325 cm^2) which were then treated as follows:

i for one portion a total count was immediately determined using the total count rinse method (Chapter 2 page 65) and thus served as the untreated control

ii a second portion was washed in anionic detergent, diluted in sterile water to 1.2% v/v as described in 2, page 66, followed by rinsing in fresh tap water before a total count was performed as above.

iii a third portion was washed and rinsed as described in ii above and was then folded and stored in a closed glass Petri dish at room temperature and 60% R.H. for 24h before a total count was performed (as above)

iv a fourth portion was washed and rinsed as described in ii above and was then folded and stored in a glass Petri dish (lid ajar) at 50°C, 30% R.H. for 24h before a total count was performed (2 page 65).

The experiment was carried out for a total of 6 cloths

5.2.2 Results

The results in Tables 15 to 17 are presented as the total counts per cm^2 of cloth as evaluated by the rinse method. In each table, the results for each treatment are shown together with the untreated control. The mean count for each treatment ($\bar{x}$) together with the sample size and standard deviation is also shown. In order to determine the effect of treatment, the means were compared using the Student's "t" test (details of test given in Appendix 2). The t and p values derived by comparing the treatment and control means using the Student's "t" test are shown in Table 18. The results in Table 15 show the high levels of contamination recovered from untreated cloths. These cloths had been in use in domestic kitchens for only 3 days and in 3 out of the 6 cloths, total counts of greater than 1000 C.F.U's/cm^2 (or 3.25×10^5 per cloth portion) were recorded. For 2 of these cloths, counts of greater than 3000 C.F.U's/cm^2 (or $>9.75 \times 10^5$ per cloth portion) were recorded. Immediately following detergent wash and rinse treatment, the mean total count was reduced to 582 C.F.U's/cm^2 compared to 1,562 C.F.U's/cm^2 for the untreated control. This treatment was found to be significant at the $p<0.1$ level (Table 18) although in one instance (cloth sample 5), the treatment resulted in an increase in total count compared to the untreated control.

The results in Table 16 show the effect of detergent wash and rinse followed by 24h storage at room temperature. Storage resulted in an increase in total counts in cloths as compared to the counts recorded immediately following detergent treatment (Table 15). The mean total count

Table 15. Total counts recovered from cloth portions treated by detergent wash and rinse

Cloth sample	Total counts per cm^2 of cloth	
	Untreated control	Detergent wash and rinse
1	651	70
2	335	84
3	3,053	474
4	≥3,177	625
5	170	757
6	1,984	1,480
mean ($\bar{x}$)	1,561.6	581.6
sample size	6	6
std. dev.	1,362.5	521.6

Table 16 Total counts from cloth portions treated by detergent wash and rinse followed by storage at R.T. for 24h

Cloth sample	Total counts per cm^2 of cloth	
	Untreated control	Detergent wash and rinse & 24h storage at R.T.
1	651	2,263
2	335	2,185
3	3,053	--
4	≥3,177	2,588
5	170	883
6	1,984	2,309
mean ($\bar{X}$)	1,561.6	2,045.6
sample size	6	6
std. dev.	1,362.5	667.4

Table 17 Total counts recovered from cloth portions treated by detergent wash and rinse followed by drying at 50°C for 24h

Cloth sample	Total counts per cm^2 of cloth	
	Untreated control	Detergent wash & rinse & drying at 50°C
1	651	3
2	335	3
3	3,053	2
4	⩾3,177	70
5	170	0
6	1,984	26
mean ($\bar{X}$)	1,561.6	17.3
sample size	6	6
std. dev.	1,362.5	27.55

Table 18. Student "t" values and probability (p) derived by comparing the treatment and control means

d.f. = degrees of freedom		
Treatment		
Detergent	d.f. =	10
wash and rinse	t =	1.64
	p =	<0.1 >0.05
Detergent	d.f. =	9
wash and rinse	t =	0.76
and 24h storage	p =	>0.10
at R.T.		
Detergent	d.f. =	10
wash and rinse	t =	2.8
and 24h drying	p =	<0.01
at 50°C		

increased from 1,562 C.F.U's/cm^2 for untreated control to 2,046 C.F.U's for detergent treatment followed by storage and statistically there was no significant difference between these counts ($p>0.1$ Table 18).

The results in Table 17 show the effect of detergent wash and rinse followed by 24h drying at 50°C. This treatment dramatically reduced total counts compared to control and other treatments although contamination was still recovered from all but one of the cloths. Counts of less than 4 C.F.U's/cm^2 were recorded in 4 out of 6 cloths and the highest count recorded was 70 C.F.U's/cm^2. Overall, the mean total count was reduced to 17 C.F.U's/cm^2 compared to 1,562 C.F.U's/cm^2 for the untreated control. This treatment was found to be significant at $p<0.01$ level (Table 18).

5.3 The effect of detergent wash followed by high temperature drying on contaminated cloths

Results from the previous experiment (page 114) indicate that even following a detergent wash and drying for 24h at 50°C, organisms are still recoverable from cloths. In order to determine whether contamination could be eliminated from cloth by drying at an elevated temperature, the experiment was repeated with a drying temperature of 80°C.

5.3.1 Method

In a method similar to that described on page 113 cloths returned to the laboratory were aseptically divided into 5 portions (of approximately 200cm^2). Each of the 5 portions was then treated as follows:

i For one portion, a total count was immediately determined using the total count rinse method (Chapter 2 page 65) and this served as the untreated control

ii A second portion was washed in anionic detergent (prepared as described in 2, page 66) followed by rinsing in fresh tap water and was then folded and stored in a glass Petri dish (lid ajar) at 80°C, <5% R.H. for 1h before a total count was performed

iii A third, fourth and fifth portion was each treated as in ii above except that they were stored at 80°C for 2h, 3h and 4h respectively

The experiment was carried out with a total of 5 cloths and results are presented for each cloth

5.3.2 Results

The results in Table 19 are presented as the total counts per cm^2 of cloth for each of the 5 cloth portions.

It can be seen that whereas drying for 1h at 80°C totally eliminated contamination from all but one of the cloths,

Table 19. Total counts recovered from cloth portions treated by detergent wash and rinse followed by drying at 80°C for various lengths of time up to 4h

Cloth sample	Total counts per cm^2 of cloth				
	Untreated control	Detergent wash & rinse followed by drying at 80°C for			
		1h	2h	3h	4h
1	1×10^5	0	0	0	0
2	8.8×10^4	<10*	0	0	0
3	3.5×10^4	0	0	0	0
4	5.5×10^4	0	0	0	0
5	2.5×10^4	0	0	0	0

* below limits of accurate counting

drying at 80°C for 2 hours or longer resulted in the elimination of contamination from all the cloths.

It should be noted that the levels of contamination for control cloths in this experiment were much higher than in the previous experiment (Table 15 page 115).

5.4 Chemical disinfection of cloths

In a final experiment in this Chapter on decontaminating cloths in the laboratory, the effect of treatment with chemical disinfectants was investigated. Cloths were examined immediately following disinfection and after disinfection and storage for 24 hours in order to look for (1) residual activity and (2) regrowth of residual survivors. The disinfectants used in this investigation were sodium hypochlorite at a dilution of 4% v/v and stericol at a dilution of 2% v/v (Chapter 2, page 66).

5.4.1 Method

In a method similar to that described on page 113 cloths returned to the laboratory were aseptically divided into 5 portions (approximately 200 cm^2) which were treated as follows:

i One portion which served as an untreated control, was immersed in 100ml sterile distilled water for 2 mins, rinsed under a cold running tap for 30s and wrung out. Cloth portions were then transferred to the neutralizing medium (Chapter 2, page 67) and serial dilutions of the neutralizing rinse fluid were prepared in quarter strength Ringers solution before the Miles and Misra total count method (Chapter 2, page 61) was carried out

ii A second portion was immersed in 100ml of 2% V/V Stericol solution as described in Chapter 2, page 66 for 2 mins. It was then rinsed under a running tap for 30s before it was transferred to the neutralizing medium and total counts determined as described above

iii A third portion was immersed in 100ml of 2% V/V Stericol solution for 2 mins. It was then rinsed under a running tap for 30s, wrung out, folded and stored in a glass Petri dish at room temperature for 24h before it was transferred to the neutralizing medium and a total count performed as described above

iv A fourth portion was immersed in 100ml of 4% V/V sodium hypochlorite solution (Chapter 2, page 66) for 2 mins. It was then rinsed under a running tap for 30s and wrung out before it was transferred to the neutralizing medium and a total count performed as described above.

v A fifth portion was immersed in 100ml of 4% V/V sodium hypochlorite solution for 2 mins. It was then rinsed under a running tap for 30s, wrung out, folded and stored in a glass Petri dish at room temperature for 24h before it was transferred to the neutralizing medium and a total count performed

The experiment was carried out with 13 cloths in all. For the last 4 of the cloths only, portions iii and v were neutralized immediately after disinfection by immersion in 100ml of neutralizing medium for 10 mins. These cloth portions were then wrung out and stored at room temperature in a glass Petri dish for 24h before the total count was performed.

5.4.2 Results

Effects of chemical disinfection on contaminated cloths are shown in Table 20 .

There were no detectable survivors in five of 13 cloths after treatment with 2% V/V Stericol. For the remaining eight cloths, although reductions were achieved, total

Table 20 Total counts recovered from cloth portions after treatment with hypoclorite 4000 ppm available chlorine and Stericol 2% v/v

Cloth sample	Total count per cm^2 cloth				
		Stericol 2% v/v		Hypochlorite 4000 ppm $AvCl_2$	
	Initial count	After treatment	After treatment and 24 h storage	After treatment	After treatment and 24 h storage
1	5.0×10^2	0	0	0	0
2	7.5×10^2	0	0	0	0
3	1.0×10^3	0	0	0	0
4	8.5×10^4	2.5×10^4	4.5×10^4	0	0
5	4.0×10^5	2.5×10^4	2.5×10^5	2.5×10^2	8.5×10^4
6	5.0×10^5	5.0×10^4	0	0	0
7	6.0×10^5	3.0×10^5	2.5×10^5	0	5.0×10^3
8	1.1×10^6	5.0×10^3	2.5×10^2	2.5×10^3	4.5×10^4
9	2.5×10^6	3.0×10^3	0	0	0

Cloth sample	Initial count	After treatment	After treatment neutralization and 24 h storage	After treatment	After treatment neutralization and 24 h storage
10	5.0×10^2	0	1.1×10^5	0	1.0×10^3
11	2.0×10^3	0	3.0×10^5	0	3.7×10^3
12	3.7×10^4	2.5×10^3	8.0×10^4	0	4.5×10^3
13	6.5×10^4	3.0×10^4	3.0×10^5	2.5×10^3	4.5×10^5

counts in excess of $10^3/cm^2$ were recorded. The results indicate that the efficiency of the disinfection procedure was related to initial contamination levels; for the 5 cloths which were satisfactorily disinfected, initial counts were of the order of 10^2-10^3 organisms per cm^2 compared with 10^4-10^6 for cloths where satisfactory disinfection was not observed. For all 9 cloths which were stored for 24h without neutralization of the disinfectant, the initial reduction was maintained over the 24h period and for 2 of these cloths a further reduction to give no detectable survivors was achieved. Where Stericol was neutralized before storage of cloths, regrowth of residual survivors was observed in all 4 cloths even though a zero count had previously been recorded in 2 of the cloths.

Using hypochlorite solution 4% V/V (4,000 ppm) results indicate no detectable survivors in 10 out of 13 cloths immediately after disinfection. For the remaining 3 cloths, although reductions were achieved, counts of 10^2-10^3 organisms/cm^2 were recorded. For the 9 cloths stored for 24h without neutralization of hypochlorite, zero counts were recorded for 6 cloths but for the remaining 3 cloths, regrowth of residual survivors was observed. For the 4 remaining cloths which were neutralized before storage, regrowth of residual survivors was observed even though a zero count had previously been recorded in 3 of the cloths.

Discussion

Although the primary aim of this chapter was not to study naturally occurring contamination on cloths, the results do confirm those of previous domestic studies (Scott *et al* 1982) indicating that cloths become heavily contaminated during use in the domestic environment. Tables 15 and 20 show initial counts of 1.7×10^2 to 2.5×10^6 cfu/cm^2 from cloths returned to the laboratory after 3 days use.

In the first part of the study detergent washing, rinsing and drying of cloths were investigated. Detergent washing and rinsing produced limited reductions in microbial contamination and where cloths were stored at room tempeature for 24h, during which time they remained damp, an increase in contamination was usually observed indicating multiplication of organisms. When the drying temperature was raised to 50°C, this produced a significant reduction in cloth contamination at 24h, but this method cannot be considered as a reliable decontamination method, since 3 of the 7 cloths showed contamination. When the drying temperature was increased to 80°C, effective decontamination was consistently achieved within 2h. These results are in agreement with those of Westwood *et al* (1971) and Walter and Schillinger (1975) who recommended laundering and adequate drying as an effective means of decontaminating wet cleaning utensils. Decontaminating cloths by means of wet heat, eg boiling, was not investigated here and although likely to be effective, is rarely practised nowadays. Many of the disposable-type cloths would probably disintegrate if subject to boiling.

Results from the second part of the study identify problems in using chemical disinfectants for decontaminating cloths and further confirm the finings of Westwood et al (1971) and Babb et al (1981). A phenolic and hypochlorite disinfectant were chosen for the study, both of which are commonly used for general disinfection of surfaces in domestic catering and other environments. Quaternary ammonium compounds were not evaluated although they are used in catering environments and have recently become available in the home. The results indicate that although hypochlorite achieved better initial results than the phenolic, producing no detectable survivors in 10 of 13 cloths, compared with 5 of 13 cloths for the phenolic, neither disinfectant can be relied upon to produce consistently satisfactory de-contamination, particularly with heavily contaminated cloths. These results indicate the potential hazard of using dis-infectants for decontamination of cloths prior to storage; for 12 of 26 cloth portions examined, storage produced regrowth of residual survivors. This occurred particularly with hypochlorite-treated cloths where residual hypochlorite would be rapidly destroyed during drying. Regrowth was observed in all cloth portions where residual disinfectant was neutralized before storage.

In neither of the investigations (detergent washing and drying or chemical disinfection) was any attempt made to detect spore forming organisms, although such organisms are known to be more resistant to heat and chemicals. The aim of the investigations was not to produce sterile cloths as this is considered neither realistic nor necessary but to identify effective decontamination methods.

From the results, it must be concluded that for effective treatment of cleaning cloths, heat must be regarded as the most reliable method. Where chemical disinfection is required for rapid decontamination, this must be done only immediately before or between cleaning activities. Chemical disinfection may not be reliable for heavily contaminated cloths, which could be given a detergent wash and rinse to reduce the initial bioburden before disinfection.

CHAPTER 6

LABORATORY STUDIES TO ASSESS THE PROBABILITY OF REDUCING THE TRANSFER OF CONTAMINATION FROM SURFACES BY THE USE OF DISINFECTANTS

6.1 Introduction

Disinfectant testing usually comprises a number of stages including "in vitro" suspension tests for primary screening, followed by laboratory evaluation of activity on contaminated surfaces and finally "in-use" tests or field studies.

Extensive reviews of disinfectant testing are given in a chapter by Reybrouck in the text on principles and practice of disinfection etc. (edited by Russell et al 1982) and by Bloomfield (1990) in a chapter on the evaluation of antimicrobial activity of disinfectants (SAB Technical Series).

Since clinical trials of disinfectants, particularly those involving some assessment of infection risks, tend to be of little value unless conducted on a very large scale, there is a tendency to rely heavily on the results of controlled laboratory tests. Over the years, surface test methods have been devised in which the disinfectant is applied to a surface which has been inoculated with a bacterial test suspension and dried at constant humidity.

The numbers of recoverable organisms are determined before and at intervals after disinfectant application. The purpose of this method of testing disinfectants is to provide information on how products (which have already been identified in "in vitro" testing as likely to be active on surfaces) perform when actually applied

to a contaminated surface. A number of standard surface tests based on this approach are operational in various European countries including Holland (van Klingeren 1983), Germany (Beck et al 1977) and France (AFNOR 1981 b). For the United Kingdom, the only standard surface test operating is the Lisboa tube test which is used for evaluation of disinfectants in the meat processing industry (Anon 1967, Blood et al 1981).

Many disinfectant tests have been carried out on environmental surfaces contaminated either artificially or under normal conditions of use. Opinions appear to remain divided as to the merits of chemical disinfectants and the effectiveness of soap and water.

Velsey and Michaelsen (1964) and Duppre (1975) showed that detergent or soap and water were equally effective as disinfectants in reducing bacterial contamination on hospital floors.

From a study carried out in 1966, Ayliffe et al reported that cleaning hospital floors with a phenolic disinfectant caused a significantly greater reduction in bacterial flora than washing with soap and water when the area was protected from recontamination. However, in a subsequent study, Ayliffe et al (1967) found that regular use of disinfectants in cleaning ward floors produced no greater reductions in the equilibrium level of bacteria on the floor than was achieved

with soap and water. On the other hand, Litsky and Litsky (1968), from another study on hospital floors, reported that low bacterial counts can be obtained when effective disinfectant-detergents are used in combination with effective housekeeping techniques. Gilbert (1970) carried out a comparison of disinfectant and cleaning procedures at worksurfaces in retail food premises and found that a "two-step" procedure involving anionic detergent and hypochlorite solutions produced the most satisfactory results in reducing bacterial counts. Ojajarvi and Makela (1974) evaluated a new washing/disinfection preparation for hospital surfaces which had a long-lasting antibacterial effect. Use of the preparation resulted in a decrease in total bacterial counts and also a decrease in the isolation fequencies of Gram negative bacilli and Staph. aureus. Scott et al (1984) reported on the development of an in-use test for domestic disinfectants which were applied to various surfaces. Single and repeated daily application tests demonstrated that hypochlorite and phenolic disinfectants can be used to produce substantial reductions in contamination levels but that the period of maximum protection afforded by disinfection is relatively brief (3-6 h). Use of detergent and water produced no overall reduction in contamination levels and actually resulted in apparent increases in contamination, possibly

due to surfactant or mechanical break-up and redistribution of cell aggregates. Hypochlorite was found to be more effective against enterobacteria then phenolic disinfectant.

As a result of laboratory tests on glass and ceramic surfaces, Werner (1975) recommended the use of broad-spectrum disinfectants. Although soap and water was found to be effective against Gram positive cocci, it was ineffective against organisms such as Klebsiella and the author stated that the exclusive use of soap and water should not be substituted for disinfection.

Much of the work described in this thesis is concerned with methods of reducing or eliminating the risk of transfer of contamination from one inanimate surface to a more critical site or surface. The purpose of the experiments described in this chapter is to attempt to determine the extent to which a range of currently available disinfectants can be used successfully to reduce or eliminate contamination transfer from surfaces and to evaluate some of the factors which may interfere with the successful use of disinfectants.

The method devised is closely based on the German DGHM method described by Beck et al (1977). The choice of the DGHM method, which uses contact plate sampling,is consistent with other assessments described in this thesis. By using a contact rather than a rinse method, the activity of the disinfectant is measured in terms of its ability to prevent transfer of contamination,

in this case by contact from a laminate surace to an agar surface. The advantages and disadvantages of contact sampling are discussed in chapter 3.

The inoculum sizes used for the work described in this chapter (a range of 10^2 - 10^6 organisms on the surface) were chosen to represent levels of contamination of surfaces which may occur during use as indicated by Gilbert (1970) and discussed in Chapter 4.

6.2 Comparison of various chemical disinfectants versus E. coli, Staph aureus and Ps. aeruginosa on worksurfaces

The first experimental section of this chapter describes an experiment designed to compare the activity of a range of commonly used disinfectants against E. coli, Staph. aureus and Ps. aeruginosa on laminate squares (representing a typical worksurface) under both clean and soiled conditions. For the purposes of this study, plasma was chosen to represent organic soil of a type which might be found in a hospital environment. A water control is included to assess the effectiveness of drying alone. From the results, a "probability of effective disinfection" is calculated. This probability is based on the likelihood of the various procedures producing a viable count of 10 or fewer transferable organisms per area of laminate square when sampled with a contact plate. For the purposes of this thesis, satisfactory disinfection is defined as less than 10 transferable organisms per sample area. This was chosen following the recommendations of Mendes et al (1978) for critical food surfaces and with regard to the infection risks posed by cross contamination as discussed in Chapter 1 page 28 and Chapter 4 page 107.

6.2.1 Method

For this experiment laminate squares as described in Chapter 2, page 63 were prepared according

to the method on page 63 .

Laboratory stains of E. coli, Ps. aeruginosa (6750) and Staph. aureus in suspensions of water or 5% v/v plasma for E. coli only and 20% v/v plasma (as described in Chapter 2, page 62) were inoculated onto laminate squares. Using a Finn Pipette, three separate 100 ul drops of each dilution were pipetted onto squares, giving total surface drop counts in the range 10^6 - 10^2 organisms. The laminate squares were dried for 1 h at 37°C.

Following drying, 100 ul drops of disinfectant solutions (as described in Chapter 2, page 66) or sterile distilled water were added to the dry inocula. After a 5 minute contact time, the drops were sampled using contact plates containing nutrient agar and 3% Tween 80, 0.3% Lecithin and 0.5% sodium thiosulphate (Appendix 1). Contact plates were held in place for 30 secs. and weighted with 200g weights. The experiment was repeated up to three times for each inoculum of test organism. The results are expressed as the number of recorded counts of 10 or less organisms per contact area out of a possible total of 9 replicate contact plates.

6.2.2 Results

The results of the evaluation of activity of disinfectants versus Staph. aureus, Ps. aeruginosa and E. coli are shown in Table 21, 22 and 23 respectively. For each table, results are given

for activity in the absence of plasma and in the presence of 20% v/v plasma. For E. coli, survival was negligible in the total absence of plasma and therefore activity in the presence of 5% v/v and 20% v/v plasma was assessed. The results indicate that disinfectant activity varies according to the test organism, the size of the test inoculum and the presence or absence of plasma.

For Staph. aureus (Table 21) in the absence of disinfectant, the organism consistently survived the effects of drying on the surface both in the presence and absence of plasma. In the absence of plasma, the chlorine - based disinfectants were slightly superior to the phenolics with 33-77% of total samples showing counts of 10 or less. Activity was inconsistent against an inoculum of 10^6 for all disinfectants and against a 10^5 and 10^4 inoculum for sodium hypochlorite and the phenolics. In the presence of 20% v/v plasma, the action of all of the disinfectants was considerably reduced giving a consistent disinfectant action against an inoculum size of 10^2 only.

For Ps. aeruginosa (Table 22) as expected, the lethal effects of drying alone were more marked in the absence of plasma than for Staph. aureus. With the addition of disinfectants, the risk of transfer of contamination was substantially reduced with 86%-100% of total samples showing a count of 10 or less. However, this activity

Table 21 Evaluation of the activity of disinfectants versus Staph. aureus on laminate surfaces

a. 0% plasma

Inoculum Size	Total no. of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	2/6	9/9	9/9	9/9	9/9
10^3	0/9	8/9	9/9	9/9	5/9
10^4	0/9	5/9	8/9	5/9	1/9
10^5	0/9	2/9	8/9	0/9	0/9
10^6	0/9	1/9	1/9	0/9	0/9
Total samples of $\leq$ 10	2	25	35	23	15
Total samples	42	45	45	45	45
Percentage of samples of $\leq$ 10	5%	55%	77%	51%	33%

b. 20% v/v plasma

Inoculum Size	Total no. of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	6/6	6/6	6/6	6/6	6/6
10^3	0/9	0/9	0/9	0/9	0/9
10^4	0/9	0/9	0/9	0/9	0/9
10^5	0/9	0/9	0/9	0/9	0/9
10^6	0/9	0/9	0/9	0/9	0/9
Total of samples of $\leq$ 10	6	6	6	6	6
Total samples	42	42	42	42	42
Percentage of samples of $\leq$ 10	14%	14%	14%	14%	14%

Table 22. Evaluation of the activity of disinfectants versus Ps. aeruginosa on laminate surfaces

a. 0% plasma

Inoculum Size	Total no. of counts of 10 or less/Total no. replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	3/3	3/3	3/3	3/3	3/3
10^3	3/3	3/3	3/3	3/3	3/3
10^4	3/3	3/3	3/3	3/3	3/3
10^5	3/3	3/3	3/3	3/3	3/3
10^6	4/9	7/9	9/9	6/9	9/9
Total samples of $\leq$ 10	16	19	21	18	21
Total sample	21	21	21	21	21
Percentage of samples $\leq$ 10	76%	90%	100%	86%	100%

b. 20% v/v plasma

Inoculum Size	Total no. of counts of 10 or less/Total no. replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	0/9	9/9	9/9	9/9	9/9
10^3	0/9	7/9	6/9	3/9	5/9
10^4	0/9	2/9	3/9	0/9	1/9
10^5	0/9	0/9	0/9	0/9	0/9
10^6	0/9	0/9	0/9	0/9	0/9
Total samples of $\leq$ 10	0	18	18	12	15
Total samples	45	45	45	45	45
Percentage of samples $\leq$ 10	0%	40%	40%	27%	33%

was again greatly reduced in the presence of 20% v/v plasma with only 27%-40% of samples showing a count of 10 or less.

For E. coli (Table 23) the organism survived the effects of drying in the presence of both 5% v/v and 20% v/v plasma (but not, as stated previously, in the total absence of plasma)

The relatively lower activity of the disinfectants in the presence of 5% v/v plasma compared with that for Ps. aeruginosa and Staph. aureus in the absence of plasma suggests that even relatively low levels of soil may result in substanital inactivation. In general, consistent disinfection action was achieved only with an inoculum size of 10^3 or less. In the presence of 20% v/v plasma, activity was further reduced and was similar to that observed for Ps. aeruginosa with 24%-48% of total samples giving a count of 10 or less.

Table 23 Evaluation of the activity of disinfectants versus E. coli on laminate surfaces

a. In the presence of 5% v/v plasma

Inoculum Size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	5/9	9/9	9/9	9/9	9/9
10^3	0/9	9/9	9/9	9/9	5/9
10^4	0/9	3/9	6/9	5/9	2/9
10^5	0/9	0/9	1/9	0/9	2/9
10^6	0/9	0/9	0/9	0/9	1/9
Total samples of $\leq$ 10	5	21	25	23	19
Total samples	45	45	45	45	45
Percentage of samples $\leq$ 10	11%	47%	56%	51%	42%

b. In the presence of 20% v/v plasma

Inoculum Size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	0/6	6/6	6/6	6/6	6/6
10^3	0/9	3/9	7/9	4/9	7/9
10^4	0/9	2/9	4/9	0/9	1/9
10^5	0/9	0/9	2/9	0/9	1/9
10^6	0/9	0/9	1/9	0/9	0/9
Total samples of $\leq$ 10	0	11	20	10	15
Total samples	42	42	42	42	42
Percentage of samples $\leq$ 10	0%	26%	48%	24%	36%

6.3 The influence of various factors on the activity of disinfectants versus Staph. aureus on surfaces

The second experimental section of this Chapter describes further experiments using the method described in the previous section in order to determine the effect of contact time and wiping upon the activity of disinfectants on surfaces. For the purpose of these experiments, Staph. aureus was chosen as the test organism due to its relative resistance both to drying and to chemical disinfectants as shown in Table 21. The tests were carried out on a different type of surface than had been used previously. Due to general wear and tear, new surfaces were purchased at the start of these experiments. These consisted of a formica material called Resopal solid grade (Resopal UK Ltd, Macclesfield). The surface of this material is a melamine-formaldehyde resin as described by van Klingeren (1978).

6.3.1 Method

A method similar to that described in the previous section (page 136) was used to determine the following:

a. The effect of a different surface type on disinfectant activity.

b. The influence of an increased (30 minutes) disinfectant contact time.

c. The influence of wiping in addition to the action of disinfectants. For this experiment, surfaces were inoculated with

Staph. aureus in 20% v/v plasma and dried in the usual way. Following upon the application of disinfectants or water, the surface was immediately wiped with a clean, sterile cloth portion (160 cm^2) (prepared as described in Chapter 2, page 64) using the following standardised method. A circular area of 25 cm^2 surrounding each drop was wiped with a fresh cloth portion with 3 wipes from left to right followed by 3 strokes from top to bottom of the circle. The drops of inoculum to which water was added and allowed to dry were not wiped and served as a comparative control to determine the effects of drying alone. Another set of inoculated drops were used to determine the relative effect of wiping alone without the addition of either water or disinfectants.

6.3.2 Results

The results of experiments to determine the influence of surface type (Table 24) indicate that the effects of both drying and disinfectant application can be affected by the nature of the surface. In the absence of disinfectants, survival on formica surfaces was greater than on laminate surfaces. The activity of disinfectants was also less on the laminate surface type (Table 21) than on the formica surface and

Table 24. Evaluation of the activity of disinfectants versus Staph. aureus on formica surfaces (5 minute contact time)

a. 0% plasma

Inoculum size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	4/9	9/9	9/9	9/9	9/9
10^3	5/9	9/9	9/9	9/9	9/9
10^4	0	9/9	9/9	8/9	9/9
10^5	0	9/9	8/9	7/9	8/9
10^6	0	7/9	9/9	7/9	7/9
Total samples of $\leq$ 10	9	43	44	40	42
Total samples	45	45	45	45	45
Percentage of samples of $\leq$ 10	20%	95%	98%	88%	93%

b. 20% v/v plasma

Inoculum size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	0/9	8/9	6/9	8/9	8/9
10^3	0/9	0/9	2/9	3/9	9/9
10^4	0/9	0/9	1/9	3/9	9/9
10^5	0/9	0/9	0/9	0/9	4/9
10^6	0/9	0/9	0/9	0/9	3/9
Total samples of $\leq$ 10	0	8	9	14	33
Total samples	45	45	45	45	45
Percentage of samples of $\leq$ 10	0%	18%	20%	31%	73%

and there was a marked variation in the action of Stericol on the 2 different surfaces.

The results of experiments to determine the influence of a longer disinfectant contact time (30 mins versus 5 mins as shown in Tables 25 and 21 respectively) indicate that contact time had little effect upon the action of the chlorine-based disinfectants and Clearsol in the absence of plasma but did bring about improved activity in the presence of plasma. For Stericol, activity both in the absence and presence of plasma was greatly increased as a result of the longer contact time.

The results of experiments to determine the influence of cloth wiping (Table 26 and 21) in the presence of 20% v/v plasma indicate that disinfectant activity can be substantially increased by the additional physical action of wiping with a cloth. The results obtained by "wiping alone" show that wiping per se contributes a large proportion of this increase in activity.

Table 25. Evaluation of the activity of disinfectants versus Staph. aureus on formica surfaces (30 min. contact time)

a. 0% plasma

Inoculum Size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	4/9	9/9	9/9	9/9	9/9
10^3	3/9	9/9	9/9	9/9	9/9
10^4	0/9	8/9	9/9	7/9	9/9
10^5	0/9	8/9	8/9	8/9	9/9
10^6	0/9	8/9	9/9	7/9	7/9
Total samples of $\leq$ 10	7	42	44	40	43
Total samples	45	45	45	45	45
Percentage of samples $\leq$ 10	15%	93%	98%	88%	95%

b. 20% v/v plasma

Inoculum size	Total no of counts of 10 or less/Total no replicates				
	Water	Sodium hypochlorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v
10^2	0/9	9/9	9/9	9/9	9/9
10^3	0/9	5/9	5/9	6/9	9/9
10^4	0/9	0/9	3/9	4/9	8/9
10^5	0/9	0/9	1/9	2/9	6/9
10^6	0/9	0/9	1/9	1/9	8/9
Total of samples of $\leq$ 10	0	14	19	22	40
Total samples	45	45	45	45	45
Percentage of samples $\leq$ 10	0%	31%	42%	49%	88%

Table 26 Evaluation of the activity of disinfectants versus Staph. aureus on formica surfaces

The influence of wiping with a cloth

a. 20% v/v plasma

Inoculum size	Total no. of counts of 10 or less/Total no. replicates					
	Water	Sodium hypoclorite 2,500 ppm	NaDCC 2,500 ppm	Clearsol 1% v/v	Stericol 2% v/v	Wiping alone
10^2	0/9	9/9	9/9	9/9	9/9	9/9
10^3	0/9	9/9	8/9	9/9	9/9	9/9
10^4	0/9	8/9	9/9	9/9	9/9	9/9
10^5	0/9	8/9	4/9	6/9	8/9	3/9
10^6	0/9	6/9	4/9	6/9	5/9	1/9
Total samples of $\leq$ 10	0	40	34	39	40	31
Total samples	45	45	45	45	45	45
Percentage of samples of $\leq$ 10	0%	88%	73%	87%	88%	69%

6.4 Discussion

The results of the experiments described in this Chapter allow some observations to be made with regards the probability of reducing the transfer of contamination from surfaces by the use of disinfectants.

Firstly, the results confirm those described in Chapter 4 and indicate that drying alone cannot be relied upon to bring about the disinfection of most particularly soiled surfaces but also of clean surfaces.

Factors which may also impair disinfectant action include not only the presence of soil and the presence of heavy contamination but also the type of surface to be disinfected, together with the age and condition of the surface and the time of contact.

Results of suspension tests have shown that the presence of organic soil reduces the activity of disinfectants (as discussed by Russell in a chapter on factors affecting the activity of antimicrobial agents: Russell, Hugo and Ayliffe 1982). The results of surface tests described here indicate that the inactivation of disinfectants (both chlorine-based and phenolics) applies not only to bacteria in suspension but also to bacteria dried onto surfaces. The results also indicate that even at 5% v/v plasma, disinfectant activity was substantially reduced and further investigations are required to determine the minimum level at which soil interferes with disinfectants on surfaces. From a practical view point, the results confirm the importance of physically removing soil before the application of a disinfectant.

The results of the investigation into contact time suggest that in the absence of soil, length of contact time has little effect on disinfectant activity. In the presence of soil, however, a significant increase in activity was associated with increased contact time. This suggests that the increased activity is largely a result of increased penetration of organic soil.

Regardless of the presence or absence of soil, the results suggest that the activity of disinfectants depends on inoculum size with activity decreasing with increasing inoculum. Results indicate that in the absence of little or no soil, disinfection (ie a reduction to 10 or less transferable organisms) was consistently achieved with inoculum sizes up to 10^3 organisms although some loss of activity was observed with Stericol against E. coli and Staph. aureus on laminate surfaces. In the presence of 20% v/v plasma soil, disinfection was only consistently achieved with a maximum inoculum size of 10^2 although this was increased to 10^4 by the application of wiping in association with the disinfectant. Gilbert (1970) suggests that surface contamination following contact with food may be in the order of 10^2-$10^6/cm^2$. It should be noted that the conditions of the experimental procedure are such that a substantial initial loss of viability occurs as a result of surface drying prior to disinfection and this may not always be the case in a practical situation.

As mentioned above, the results suggest that the action of wiping with a cloth in conjunction with a disinfectant can improve disinfectant activity and that wiping alone can represent substantial "disinfection action". These results confirm those of the "in-use" assessment of cloths discussed in Chapter 9, in which it was found that a disinfectant-impregnated cloth could be successfully used to reduce contamination transfer.

One of the problems of using controlled laboratory tests to evaluate disinfectant products is the extent to which the results of these tests may or may not indicate activity under "in-use" conditions. It is difficult to determine the extent to which the results of suspension tests may correlate with the results of standard laboratory surface tests and the extent to which laboratory surface tests in turn correlate with practical in-use conditions.

Results of suspension tests (Bloomfield and Scott unpublished) based on the European Suspension Test (BS DD 1988) carried out with Staph. aureus in 20% v/v plasma soil (instead of albumin) are shown in Table 27 For a product to pass the European Suspension Test it must be shown to give a 5 log reduction (ie Micro-biocidal Effect or ME value of 5) in viable count within 5 minutes. The results in Table 27 indicate that all four disinfectant products consistently gave a greater than 6 log reduction in viable count in 5 replicate tests in the presence of plasma.

Table 27 Comparison of results from suspension and surface tests using Staph. aureus

Log Reductions (ME Values) in the presence of 20% v/v plasma and 5 mins contact time

** Suspension Test		*** Surface Test	
NaOCL (2,500 ppm)	>6	NaOCL (2,500 ppm)	0.7
NaDCC (2,500 ppm)	>6	NaDCC (2,500 ppm)	0.8
Stericol (1% v/v)	>6	Stericol (2% v/v)	3.4
Clearsol (0.625% v/v)	>6	Clearsol (1% v/v)	1.5

** = value obtained from 5 replicate tests
*** = value obtained from 9 replicate tests

ME Values = $\log (Nc \times 10^{F}) - \log ND$

Where

NC = Colony counts from "water control plates"

F = Difference in log stages between inoculum size used for NC and ND determinations

ND = Colony counts "disinfectant treated" plates

Ref: Bloomfied and Scott (unpublished)

The DGHM test (Beck et al 1977) describes a method for calculating ME values for activity of a disinfectant over and above that achieved by drying (see Table 27). Using this method, ME values were calculated for data obtained from tests on formica surfaces against Staph. aureus (as given in Table 24). Whereas suspension tests indicate consistent ME values of greater than 6, the ME values obtained in the surface tests were much lower. It should be noted that the pass criteria for the DGHM test involves a log reduction of 5 with a contact time of 30 mins (Reybrouck, personal communication).

Although the ME values can be used to compare, under controlled conditions, activity of a range of disinfectants under a variety of conditions, such values are not easily correlated with the effectiveness of a product in preventing transfer of contamination in practice. There is little information available on the relationship between a log reduction and reduced transfer of contamination.

Overall, the results from tests on surfaces described in this Chapter indicate the extent to which chemical disinfectants and chemical disinfectants together with cloths may be used to reduce the risk of transfer of contamination under controlled laboratory conditions.

The work described in this Chapter represents preliminary studies under a limited range of conditions. Further factors which may have an effect and which require investigation include the method of inoculation of surfaces, the rate and temperature of drying of contaminated surfaces, the effects of small amounts

of soiling and the effect with mixed cultures.

Ideally, as a final stage of disinfectant screening, products should be tested against naturally occurring contamination.

In an earlier field study in the home (Scott et al 1984) using phenolics and hypochlorites, the frequency of occurrence of "clean sites and surfaces" (less than 10 organisms per contact area) was determined. From the results it was estimated that by using hypochlorite together with a cloth, the probability of significant contamination transfer can be reduced from 0.8 to 0.25 and by using a phenolic and cloth, from 0.8 to 0.6. This study showed that disinfectants can be used to reduce the risk of contamination and contamination transfer at sites in kitchens, bathrooms and toilets but that chemical disinfection, by definition, does not guarantee freedom from contamination risk.

Further field studies involving the use of chemical disinfectants are described in Chapter 8 for toilets and wastetraps, in Chapter 9 for cloths and food preparation surfaces and Chapter 5 for disinfection of naturally contaminated cloths.

CHAPTER 7

FIELD AND LABORATORY STUDIES ON THE SURVIVAL AND TRANSFER OF CONTAMINATION FROM TOILETS AND WASTETRAPS

7.1 Introduction

7.1.1 Contamination of toilets

A survey of the literature reveals a limited number of references to studies on the microbiological contamination of toilets.

In a study of the microbiology of hospital toilets, Newsom (1972) found that contamination by faecal bacteria was low and he therefore considered that hospital toilets were an unlikely source of infection unless grossly soiled.

By contrast, Mendes and Lynch (1976) found that public toilets were heavily contaminated with faecal bacteria, including E. coli, Streptococcus faecalis and Proteus spp. A detailed analysis revealed that approximately 20% of toilet seat samples and 30% of WC water samples yielded colony counts of greater than 10^3 per cm^2 and that 68% of seat samples and 38% of WC water samples showed contamination with faecal bacteria.

A survey of the microbiological contamination of domestic toilets carried out by Finch et al (1978) concluded that domestic toilets were generally not heavily contaminated and that few gram negative bacilli were present. However, in a much larger survey of domestic toilets reported by Scott et al (1982), it was found that although counts were generally not high, over 16% were found to be contaminated with E. coli.

7.1.2 Studies on the transfer of contamination from toilets

Several studies have been carried out to assess the potential for transfer of contamination from toilets to surrounding sites. Darlow and Bale (1959), Bound and

Atkinson (1966) and Gerba et al (1975) carried out tests which showed that the flushing of toilets can produce a bacteria laden aerosol which may be widespread and result in the contamination of surrounding surfaces. The production of such an aerosol may be of even greater significance when the results of survival studies performed by Newsom (1972) are considered. These showed that Shigella can survive in faeces and water for 3 days, E. coli for 8 days and Salmonella for 12 days.

In considering the method of spread of sonne dysentery, Hutchinson (1956) demonstrated that toilet seats become contaminated with Sh. sonnei when heavily infected loose bulky stools were flushed away. Further, Thomas and Tillett (1973) described the role of poor conditions in junior school toilets in aiding the spread of sonne dysentery amongst pupils.

7.1.3 Contamination of wastetraps

Studies on the microbiological contamination of wastetraps have been carried out in hospitals, catering establishments, public washrooms and the home.

As discussed in the introduction (page 42) Kohn (1967) concluded that both sinks and wastetraps can act as reservoirs of Ps aeruginosa and thereby become a source of cross-contamination.

In a comparison of Ps. aeruginosa contamination in hospital and domestic environments, Whitby and Rampling (1972) sampled 114 domestic wastetraps which yielded only 7 positives for Ps. aeruginosa whereas 123 hospital wastetraps samples yielded 73 positives. As a result of this study, they concluded that contaminated wastetraps and sinks

in hospitals can play a part in cross-infection and can be a particular risk for susceptible patient populations, for example, in premature baby units, burns units or where immunosuppressive and cytotoxic drugs are used. As detailed in Chapter 1 (page 49) Ps. aeruginosa has also been found to be widely distributed in the environment of hospital pharmacies, particularly at moist sites including sinks and drains (Baird et al 1976). Strains of Ps. aeruginosa similar to those isolated from the environment were also isolated from pharmaceutical preparations.

In a survey of the bacteria present at various positions in public washrooms and toilets (Mendes and Lynch 1976), it was found that approximately 80% of washbasins overflows yielded colony counts of greater than 10^3 per cm^2 and included faecal bacteria such as E. coli and Strep. faecalis as well as Ps. aeruginosa. In a subsequent survey of kitchens (Mendes et al 1978), 69% of sink wastetraps contained faecal bacteria. The authors commented that when sinks are filled to the overlow, such contamination can easily be transferred to the fresh washing water and its contents.

Wastetraps were also found to be heavily contaminated in the domestic environment (Scott et al 1982). High colony counts were recorded for 56% of kitchen sink wastetraps and 71% of bath and basin wastetraps. E. coli was isolated from 39% of sink wastetraps; similar results for E. coli contamination in and around domestic sinks prompted Finch (pers. comm. 1981) to state that he believed E. coli to be free-living in these areas. As additional

evidence he quoted the rapidity with which new sinks were colonised with E. coli, the ease of isolation over a long period and the speed with which populations increased after being reduced to very low numbers by disinfection.

7.1.4 Studies on the transfer of contamination from wastetraps

In addition to the circumstantial evidence for the transfer of contamination from wastetraps quoted by Kohn (1967), Whitby and Rampling (1972), Baird et al (1976) and Mendes et al (1978) as referred to in the previous Section 7.1.3 , specific laboratory studies have also been performed in order to assess the potential for the spread of contamination caused by splashback from wastetraps. Kohn (1967) seeded sink wastetraps with Serratia marcescens and as a result of splashback caused by a running tap was able to recover the test organisms from the hands of people using the sink. Thomas et al (1972) also carried out 'splashback tests' as referred to in Chapter 1 (page 42) and concluded that a potential danger does exist as a result of splashback.

7.2 Field studies on the survival and transfer of contamination from toilets and wastetraps

The first section of this Chapter describes experiments carried out in the field to determine the levels and nature of contamination occurring naturally in water from hospital and institutional toilets and domestic wastetraps and on surrounding hard surfaces. The results of this study provided details of normal levels of contamination which were then used as a basis for comparison with studies involving disinfectant procedures (Chapter 8). The methods of sampling, enumeration and identification of bacterial species described in this Section (page 161) were used as a basis for further experiments described in Chapter 8.

Detailed studies were carried out on toilet contamination whereas preliminary studies only were performed on wastetrap contamination. This reflects the greater potential infection risk associated with institutional toilets in that heavy use of toilets brings many people (including compromised patients, children and the elderly) into direct contact with the toilet itself and surrounding contact sites such as handles.

A total of 12 toilets (male and female) were monitored, six of these were at Chelsea College and six in St. Stephens Hospital, London. The College units consisted of two male and two female toilets situated in the College buildings and used throughout the day, plus two male/female shared toilets in a residential hall. These were used mostly in the morning and evening and at weekends.

The hospital units consisted of three toilets serving individual side wards (general and surgical) and a further three toilets serving a 16 bed mens' ward (general, surgical and some geriatric). Studies on the contamination of water in wastetraps involved one domestic kitchen sink wastetrap only.

7.2.1 Methods

7.2.1.1 Sampling Sites and methods of sampling

7.2.1.1.1 Toilets

Toilet samples were taken from the water in the bowl, the bowl surface and rim, the seat and handle, the floor and the air.

Sampling procedures were based on a previously published method (Scott et al 1981). Flat surfaces were sampled by placing blood agar contact plates (25cm^2) in contact for 10s. Awkward surfaces such as toilet handles and rims were sampled by nutrient agar contact slides (5cm^2) (Tillomed Ltd., Henlow). Serum-coated swabs (Exogen Ltd.) pre-moistened with one quarter strength Ringer's solution were also used to sample areas of approximatley 50cm^2 adjacent to the contact sample area. The swabs were returned immediately to their plastic containers. Toilet bowl water samples (up to 30ml) were collected by pipette and transferred to sterile 25ml screw-capped bottles.

Air was sampled by exposing blood agar settle plates (Tissue Culture Services Ltd., Slough) for a period of approximately 4h.

Samples were returned to the laboratory in an insulated cool box within 1h of collection. Swabs were streaked onto blood and MacConkey agars. (Tissue Culture Services Ltd., Slough). A series of dilutions of toilet bowl

water samples were prepared using one quarter strength Ringer's solution and 0.5ml volumes of the appropriate dilutions were spread onto blood and MacConkey agar. All plates and slides were incubated aerobically at 37°C for 24 h.

7.2.1.1.2 Wastetraps

Wastetrap samples were taken from the water in the wastetrap and from the surrounding sink surface.

Wastetrap water samples (20ml) were collected by pipette and transferred to sterile 25ml screw-capped bottles.

The sink surface was sampled by placing MacConkey agar contact plates (2) in contact for 10s on either side of the wastetrap.

Samples were returned to the laboratory within 2h of collection. A serial dilution of the wastetrap water was prepared for a Miles and Misra surface viable count (page 61) on MacConkey agar. A loopful of wastetrap liquid was streaked onto MacConkey agar for identification of Gram negative organisms. All plates were incubated aerobically at 37°C for 24h.

7.2.1.2 Enumeration and identification of bacteria

Total viable counts from toilet bowl water samples were made by counting colonies on blood agar spread plates.

Total viable counts of Gram negative bacilli from toilet bowl water samples and wastetrap water samples were made from MacConkey agar spread plates and surface viable plates respectively. Total viable counts from toilet surface sites and air samples were made from colony counts on contact plates, slides and settle plates.

Total viable counts of Gram negative bacilli from the sink surface surrounding the wastetrap were made from

colony counts on MacConkey agar contact plates (average count from 2 plates).

For the identification of individual species the colonial morphology, Gram staining reactions and A.P.I. 20E reactions etc. (Chapter 2 page 60) were determined for all isolates from blood and MacConkey Petri dishes, contact plates and slides.

7.2.1.3 Sampling programme

7.2.1.3.1 Toilets

Each toilet was sampled over a period of two weeks during which time, domestic staff were requested to clean toilets daily (early morning) without application of disinfectant. In the hospital toilets, an anionic detergent product (Reckitt & Colman Ltd. Hull) was used for daily cleaning whilst in the College toilets, no product was used.

Except for air which was sampled by settle plates left in place for approximately 4h on 2 days per week (giving a maximum of 24 samples), all other sampling was carried out twice a day (am and pm) on 2 days per week (giving a maximum of 48 samples from each site).

7.2.1.3.2 Wastetraps

The wastetrap and sink surface were sampled over a period of 2 weeks during which time, no disinfectant or cleaning products were applied to sink or wastetrap. Sampling was carried out once a day (am) on 4 days per week, giving a maximum of 8 samples each for wastetrap and sink.

7.2.2 Results

7.2.2.1 Toilets

Analysis of results for male and female toilets indicated no differences and results for all toilets are therefore combined.

7.2.2.1.1 Colony counts from toilet bowl water and surrounding sites

Table 28 shows the cumulative frequency of occurrence of colony counts of 12,000, 1,000, 600, 100, 10, 1 or more per ml of bowl water expressed as a percentage of samples taken. Bowl water counts from hospital toilets were higher than those from college toilets and, for the latter, no attempt was made to differentiate counts greater than 1000/ml.

As mentioned above, hospital bowl water samples were more heavily contaminated than college samples. In the hospital, 79% of samples had counts of 1,000 or more/ml and 43% of samples had counts of 12,000 or more/ml, whereas in the college toilets, only 22% of samples had counts of 1,000 or more/ml.

Table 29 shows the results obtained from sites other than bowl water.

Despite differences in contamination levels in toilet bowl water, the hard surfaces in and around the toilet showed similar levels of contamination for both hospital and college. An exception to this was the toilet handle: 22% of samples in the hospital had counts of 100 or more compared to only 6% of college samples. The counts from settle plates for air samples also showed marked differences with 78% of college samples giving counts of 100 or more compared with 16% of hospital samples.

7.2.2.1.2 Bacterial species isolated from toilet and surrounding sites

The range of aerobic bacteria which were isolated from college and hospital toilets and identified are listed in Table 30 . Overall, the range of species isolated was similar to that found by other investigators

Table 28 Colony counts in toilet bowl water samples from college and hospital toilets over a 2 week period of sampling

Cumulative frequency of occurrence as a percentage of samples taken		
Colony counts per ml of toilet water	College	Hospital
12,000 or more	-	43
1,000 or more	22	79
600 or more	27	83
100 or more	49	95
10 or more	96	95
1 or more	100	95
Total number of samples	45	42

Table 29 Colony counts at individual sites (other than water) for college and hospital toilets over a 2 week period of sampling

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken											
	Bowl surface		Rim		Seat		Handle		Floor		Air	
	*C	*H	*C	*H	*C	*H	*C	*H	*C	*H	*C	*H
100 or more	54	47.5	46	36	26	22	6	22	92	88	78	16
10 or more	93	90	76	44	85	67	42	78	100	98	96	100
1 or more	98	97.5	89	79	100	95	86	100	100	98	100	100
Total number of samples	46	41	45	40	46	41	37	42	40	42	23	19

*C - College *H - Hospital

Table 30 Species of bacteria isolated and identified from college and hospital toilets

Escherichia coli	
Citrobacter freundii	Other Enterobacteria
Citrobacter spp.	
Enterobacter cloacae	
Enterobacter agglomerans	
Proteus mirabilis	
Klebsiella pneumoniae	
Klebsiella ozaenae	
Streptococcus faecalis	Enterococci
Pseudomonas aeruginosa	
Ps. maltophilia	Other 'pseudomonads'
Ps. cepacia	
Ps. fluorescens	
Ps. fluorescens gp.	
Pseudomonas spp.	
Pseudomonas/Alkaligenes gp.	
Acinetobacter calcoaceticus	
Aeromonas hydrophila	
α and β heamolytic streptococci	Streptococci
Staphylococcus aureus	
Micrococci	
Gram positive bacilli	

(Newsom, 1972; Mendes and Lynch, 1976). The most frequently isolated species were Gram positive bacilli and micrococci but a proportion of samples both from the toilet and surrounding areas showed the presence of one or more opportunist pathogens of enteric origin including E. coli and other entero-bacteria (species of Citrobacter, Enterobacter, Proteus and Klebsiella), and Ps. aeruginosa.

For further consideration, the bacteria listed in Table 30 were grouped together as shown. These groupings (E. coli, other Enterobacteria, enterococci, Ps. aeruginosa, other 'pseudomonads' Streptococci, Staph. aureus, Micrococci, Gram positive bacilli) were used in all subsequent tabulations of results.

The frequency of occurrence of these groups of bacterial species isolated from the toilet and surrounding sites is presented in Table 31. For the purposes of presentation of bacterial species, the sites are divided into two groups, namely, toilet sites (water, bowl surface and rim) and surrounding sites (seat, handle, floor and air).

The frequent isolation of Ps. aeruginosa from hospital but not from college toilets is in agreement with the findings of other workers (Whitby and Rampling 1972).

On MacConkey agar, individual counts of up to 4.8×10^4 and 1.2×10^4 per ml. for Ps. aeruginosa and E. coli respectively were obtained from some toilet water samples. Streptococci and species of pseudomonads other than Ps. aeruginosa were also isolated from a substantial number of college and hospital toilets whilst Staph. aureus was found in 2 samples only taken from

Table 31. Frequency of occurrence of bacterial species isolated at toilet and surrounding sites in college and hospital toilets over a 2 week period of sampling

	Frequency of occurrence			
	College		Hospital	
	*Toilet sites	*Surrounding sites	*Toilet sites	*Surrounding sites
E. coli	33	8	8	9
Other enterobacteria	54	2	50	14
Enterococci	11	20	1	11
Other streptococci	20	28	1	24
Ps. aeruginosa	11	0	58	8
Other pseudomonads	39	54	28	15
Staph. aureus	2	0	0	0
Micrococci	92	131	39	134
Aerobic bacilli	83	83	39	84
Total number of samples	136	146	123	144

*Toilet sites - water, bowl surface and rim *Surrounding sites - seat, handle, floor and air

college toilets. E coli was isolated more frequently from college toilet sites than hospital toilet sites, a reverse of the pattern observed for Ps. aeruginosa.

7.2.2.2 Wastetrap

7.2.2.2.1 Colony counts from the wastetrap water and sink surfaces

Colony counts of Gram negative bacilli per ml of wastetrap water and counts per 25 cm^2 contact area of sink surface samples over 2 weeks, are shown in Table 32 .

As might be expected, given the stagnant nature of waste-trap water and the amount of organic material likely to be collected in a wastetrap, high counts of Gram negative organisms were recorded from wastetrap water with only 1 out of 8 samples giving a count of less than 100,000/ml. On all but one occasion, the contact plate samples taken from the surrounding sink surface gave confluent (and therefore uncountable) growth (recorded as 'too numerous to count'/TNTC).

7.2.2.2.2 Bacterial species isolated from the wastetrap and sink surface

The frequency of occurrence of Gram negative bacilli isolated from the wastetrap water and surrounding sink surface is given in Table 33.

The spectrum of Gram negative bacilli isolated from sink surface and wastetrap water is similar to that found in a previous domestic study (Scott 1981) except for the absence of species of Klebsiella. This may be related to the relatively small sample size used in this investigation.

Table 32 Colony counts of Gram negative bacillia in wastetrap water samples and from sink surface contact samples over a 2 week period of sampling

Sample days	Colony counts per ml of wastetrap water	Colony counts per $25cm^2$ of sink surface
1	1.3×10^5	TNTC
2	3.9×10^6	TNTC
3	6×10^5	TNTC
4	1.6×10^5	TNTC
7	1.2×10^4	33
8	7.75×10^6	TNTC
9	5.6×10^5	TNTC
10	2.5×10^6	TNTC
Total number of samples	8	8

Table 33 Frequency of occurrence of Gram negative bacilli isolated from wastetrap water and sink surface over a 2 week period of sampling

	Frequency of occurrence	
	Wastetrap water	Sink surface
E. coli	1	0
Ent. cloacae	3	4
C. freundii	4	3
Ps. fluorescens gp.	2	0
Pseudomonas spp.	1	1
(poss. Ps. alcaligenes)	1	1
Total number of samples	8	8

7.3 Laboratory studies on the survival of naturally occurring contamination in toilet water and wastetrap water samples

As stated in the introduction to this chapter (page 158) there are suggestions that potential pathogens may be either free living and/or capable of regrowth in wet areas such as wastetraps and toilets, particularly where there is an accumulation of stagnant water.

In this section, a simple laboratory experiment is described which was devised in order to determine the potential of toilet bowl water and wastetrap water to support the survival and growth of naturally occurring contamination over a 24 hour period.

7.3.1 Method

Samples of water (10ml) from domestic toilets and kitchen wastetraps were collected by pipette and transferred to sterile screw-cap bottles. Samples were returned to the laboratory and stored at room temperature for 24 hours. Serial dilutions of all samples were prepared immediately (time 0) and at 1 hour, 2 hours, 3 hours, 4 hours, 5 hours and 24 hours after return to the laboratory and plated onto overdried tryptone soya agar (Miles and Misra method, page 61).

7.3.2 Results

Colony counts per ml for toilet and wastetrap samples are given in Tables 33 and 34 respectively.

Results for toilet water samples (Table 34) indicate that all 10 samples were contaminated with initial colony counts ranging from 10^2 - 10^6/ml. Over 24 hours counts increased so that all samples gave counts of between 10^5 - 10^6/ml.

Table 33 Colony counts of naturally occurring contamination per ml of toilet water

Replicate	Sample Time						
	0	1h	2h	3h	4h	5h	24h
1	$2.5x10^6$	$2.1x10^6$	$1.8x10^5$	$1.6x10^5$	$1.4x10^5$	$1.2x10^5$	$2x10^6$
2	$2.3x10^3$	$1x10^3$	$7x10^2$	$6.5x10^2$	$6x10^2$	$5x10^2$	$7x10^2$
3	$1x10^2$	$4x10^5$	$4x10^4$	$8x10^2$	$1x10^3$	$1x10^4$	$3x10^5$
4	$2x10^4$	$2x10^3$	$1.3x10^5$	$6x10^4$	$1x10^2$	$2.7x10^4$	$6x10^6$
5	$4.5x10^5$	$3x10^7$	$1x10^5$	$4.5x10^5$	$5x10^2$	$3.5x10^5$	$5x10^6$
6	$1x10^2$	$9x10^8$	$8x10^6$	$3x10^5$	$7x10^4$	$5x10^4$	$6x10^6$
7	$2x10^4$	$6x10^3$	$5.5x10^3$	$4.8x10^3$	$3x10^3$	$2.7x10^3$	$7x10^6$
8	$2.3x10^4$	$5.3x10^3$	$5x10^3$	$4.7x10^3$	$3.2x10^3$	$3x10^3$	$6x10^6$
9	$1x10^2$	$1x10^7$	$5.4x10^6$	$2x10^6$	$2x10^5$	$1.3x10^5$	$5.5x10^6$
10	$1x10^2$	$6x10^7$	$5x10^8$	$2x10^6$	$4.6x10^6$	$2.5x10^6$	$5x10^6$

Table 34. Colony counts of naturally occurring contamination per ml. of wastetrap water

Replicate	Sample Time						
	0	1h	2h	3h	4h	5h	24h
1	$2x10^6$	$2.4x10^5$	$2.3x10^5$	$1.9x10^5$	$1.3x10^5$	$4x10^5$	$2x10^6$
2	$4.6x10^6$	$3.8x10^6$	$4.3x10^5$	$3.7x10^5$	$3.1x10^5$	$2.8x10^5$	$6.2x10^6$
3	$3x10^6$	$2.8x10^6$	$2.2x10^6$	$2x10^6$	$2.9x10^6$	$4.2x10^6$	$1x10^7$
4	$3.8x10^6$	$3.5x10^6$	$2.8x10^6$	$2.1x10^6$	$4x10^6$	$4.8x10^6$	$2x10^7$
5	$3x10^6$	$2.8x10^6$	$2.5x10^6$	$2.4x10^6$	$3.5x10^6$	$4x10^6$	$3x10^7$
6	$3.2x10^6$	$3 x 10^6$	$2.5x10^6$	$2.2x10^6$	$3.8x10^6$	$4.2x10^6$	$4.5x10^7$
7	$8x10^8$	$4.3x10^8$	$2x10^7$	$1.3x10^7$	$2.8x10^7$	$4.5x10^7$	$1.5x10^8$
8	$2.9x10^7$	$2.7x10^7$	$2.5x10^7$	$1.5x10^7$	$4x10^7$	$4.2x10^7$	$6x10^7$
9	$7x10^8$	$6.5x10^8$	$1.5x10^7$	$1.2x10^7$	$2.5x10^7$	$4.3x10^7$	$2x10^8$
10	$3.3x10^7$	$2.3x10^7$	$1.9x10^7$	$1.8x10^7$	$4.1x10^7$	$5x10^7$	$8x10^7$

Over the first hour of sampling some large and inexplicable increases in counts were recorded. Possibly, some sample contained residual amounts of toilet cleaning and disinfecting chemicals causing the initial counts to be abnormally low.

Results from wastetrap water samples (Table 34) again show that all 10 samples were contaminated, with initial counts ranging from 10^6 - 10^8/ml. Over 24 hours, all counts remained high (10^6 - 10^8/ml) with some showing either small increases or decreases. Initial counts from wastetraps were higher than those from toilets and remained so up to 24 hours.

Further investigations with samples of wastetrap and toilet water indicated that the contamination was mainly Gram positive bacilli, micrococci and pseudomonads. In some samples E. coli and Ent. cloacae were found but survived for only 24 hours. In one sample, E. coli not only survived but grew to be the predominant species over a period of 9 days.

7.4 Discussion

Bacteriological sampling of hospital and college toilets as described in Section 7.2.2.1 indicate that a significant proportion of sites were contaminated with substantial numbers of bacteria even where toilets were cleaned on a daily basis. Although the isolation of primary enteric pathogens such as shigellas and salmonellas would not be expected in a survey of this size which represents a relatively small number of samples, opportunist Gram negative pathogens such as Ps. aeruginosa, E. coli and other enterobacteria were quite frequently isolated both from the toilet itself and from sites such as the toilet seat and handle. In the hospital, E. coli and Ps. aeruginosa were isolated from 6.5% and 47% of toilet sites respectively and in the college, E. coli and Ps. aeruginosa were isolated from 24% and 8% of toilet sites respectively. The range of organisms isolated in this study is similar to that described for public toilets by Mendes and Lynch (1976).

In general, contamination was more extensive in college and hospital toilets than had previously been found for domestic toilets. Overal 54% of bowl water samples examined in this study had counts of 600 or more organisms per ml (compared with 10.8% of domestic samples with counts of greater than 100, Scott 1981) whilst contamination levels of 100 or more organisms per 25cm^2 sample area were found on 51% of bowl surface samples (compared with 8.5% of domestic samples, Scott 1981).

Apart from contamination in the toilet itself, con-

tamination of surrounding areas may occur due to direct shedding from toilet users and/or splashing or generation of bacteria-laden aerosols during toilet flushing. Results indicated that 44% of toilet seat, handle, floor and air samples had counts of 100 or more organisms per sample area, whilst results from individual toilets indicated a relationship between the occurrence of species such as E. coli, Enterobacter and Ps. aeruginosa in the toilet itself and their isolation from surrounding areas.

From previous investigations of the domestic environment (Finch, pers. comm and Scott 1981), it was suggested that organisms such as E. coli survive and may proliferate at wet sites such as sinks to form persistent free living reservoirs of those species. The laboratory study on stagnant water indicated that toilet bowl water has the ability to support the growth and survival of bacteria. From a detailed examination of species isolated from individual toilets over the sampling period, there was little evidence to suggest that a particular species of bacterial contaminant persisted in an individual toilet for any length of time. It must be concluded that in practice, the action of flushing probably prevents contamination from persisting.

Different studies have found that levels in toilets vary. Overall, it would seem that public, institutional and hospital toilets are likely to be more heavily contaminated than domestic toilets. These variations are probably the result of several different factors including the frequency of use of the toilet, the frequency of cleaning and the type of cleaning involved.

The survey of a wastetrap and surrounding sink surface as described here confirmed (as found by Kohn 1967, Mendes and Lynch 1976, Mendes et al 1978 and Scott 1981) that wastetraps are frequently contaminated with large numbers of bacteria which may include potentially pathogenic Gram negative organisms. In this study the same species were isolated from both the wastetrap liquid and from the surrounding sink surface on a number of occasions although in this situation, it is impossible to determine whether contamination in the wastetrap originated in the sink or whether contamination of the sink occurs by splashback from the wastetrap. The potential for splashback from the wastetrap has been described by Kohn 1967 and Thomas et al 1972.

On occasions, an individual species was isolated from sink surface or wastetrap over several days suggesting that the organisms were not only surviving but also proliferating to form permanent reservoirs at those sites. Laboratory investigation confirmed the ability of stagnant wastetrap water to support the growth and survival of high levels of contamination.

CHAPTER 8

FIELD STUDES ON THE CHEMICAL DISINFECTION OF TOILETS AND WASTETRAPS

8.1 Introduction

8.1.1 Disinfection of toilets

Whilst the literature reveals a limited number of references to studies on the contamination of toilets, there appears to be even fewer references to studies on the disinfection of toilets.

Hambraeus and Malmborg (1980) evaluated different disinfection and cleaning routines for hospital toilets and although only the toilet seat was sampled, they found that the most effective of 4 different cleaning routines was disinfection after each patient. It was also found that cleaning with a detergent le d to a 4-6 fold rise in contamination. Scott et al (1984) observed that the low levels of contamination associated with the domestic toilet both before, as well as after disinfection, indicated the relative efficiency of flushing as a means of controlling infection hazards associated with the domestic toilet.

8.1.2 Disinfection of wastetraps

As a result of concern generated by the potential risk for cross-infection posed by wastetraps, especially in hospitals, attempts have been made to find a method of reliable disinfection for wastetraps.

Kohn (1970) described a relatively simple and effective method of eliminating wastetrap contamination based upon heating the water in the trap to boiling point, either by steam or by electricity. Tests performed using an experimental trap unit, heavily contaminated with Ps.aeruginosa, Staph. py ogenes and E. coli proved that, after heating for 10-15 minutes, all vegetative forms could be completely eliminated. Thomas et al (1972) found that contamination could be eliminated

from wastetraps in operating theatres by the daily addition of neat Hycolin (40ml). Previously the use of either 2% hypochlorite or 4% Hycolin solutions had proved ineffective. Baird et al (1977) reduced the number of Ps. aeruginosa isolated from hospital pharmacies by introducing environmental controls, including electric sterilising elements in wastetraps. In the domestic environment, Scott et al (1984) found that chemical disinfection of wastetraps could be effective but very short lived, that is, contamination levels were found to have returned almost to pre-treatment levels within 3 hours.

8.2 Methods

The results from the previous Chapter established the levels and types of contamination which may be found in hospital and college toilets. Using the established pattern as a comparative control, this section describes experiments designed to investigate the relative effectiveness of chemical disinfection of toilets, in particular, the effectiveness of a daily application of disinfectants and the use of a continuous release disinfectant system based on the chlorine-releasing agent tricholoroisocyanuric acid in reducing or eliminating microbial contamination from toilets.

Preliminary studies of the effectiveness of a continuous release disinfectant system (also based on trichloroisocyanuric acid) applied to wastetraps were also made.

Control experiments described in the previous Chapter and disinfectant experiments described in this Chapter were carried out sequentially.

8.2.1 Sampling sites and method of sampling, enumeration and identification of bacteria

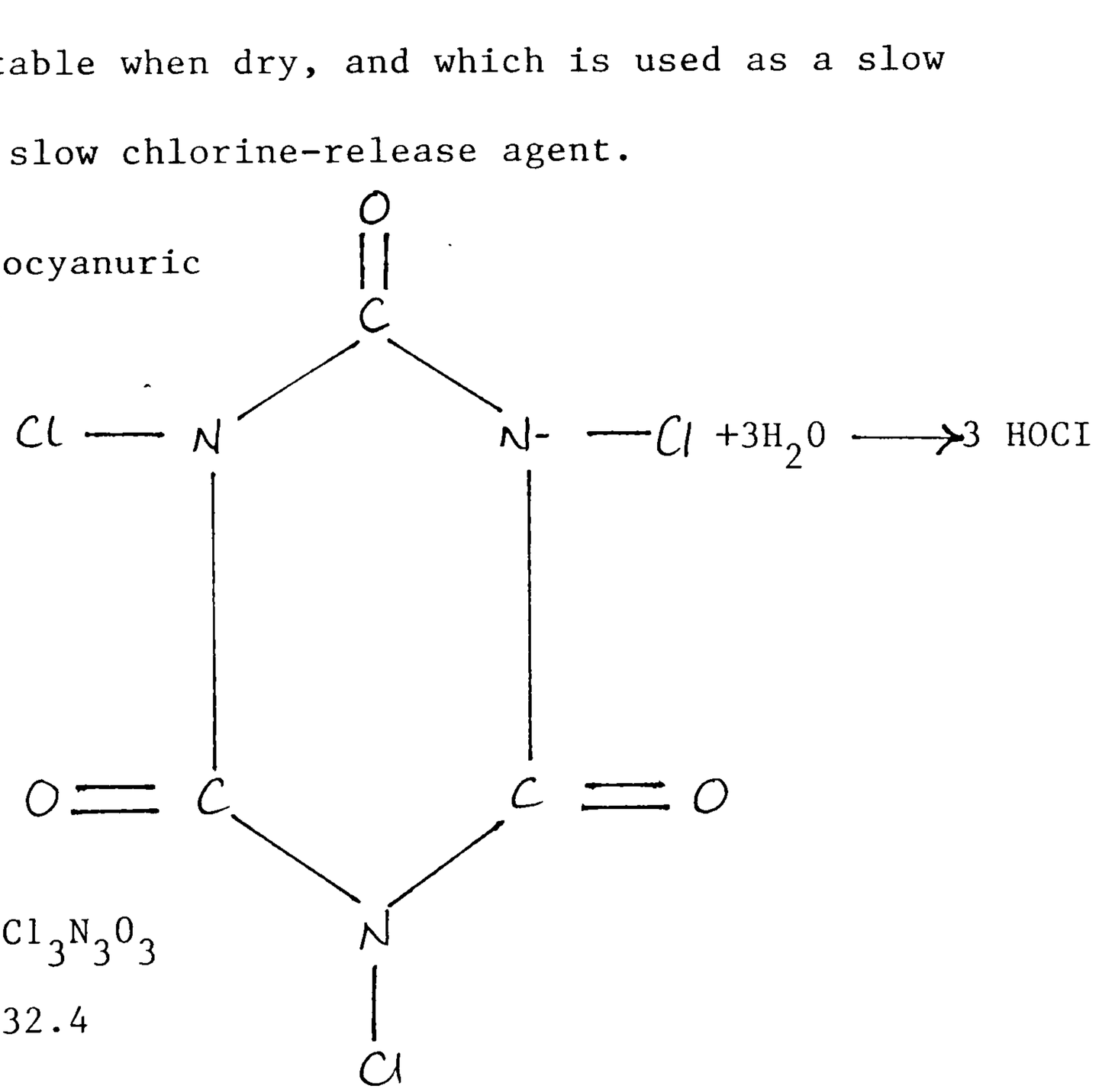
The toilet and wastetrap sites and the methods of sampling, enumeration and identification as described in the previous Chapter (Pages 161 to 163) were also used in this Chapter.

8.2.2 Disinfectant products and procedures

8.2.2.1 Toilets

The continuous release disinfectant was a solid block trichloroisocyanurate formulation packaged to release a measured dose of approximately 4ppm available chlorine into the toilet bowl with every flush (brand name 'Bleachmatic', Reckitt and Colman Ltd., Hull). Trichloroisocyanuric acid (an organic chlorine donor) is a solid compound which is stable when dry, and which is used as a slow dissolving slow chlorine-release agent.

Trichloroisocyanuric acid



Empirical formula: $C_3Cl_3N_3O_3$

Mol. wt.: 232.4

The trichloroisocyanurate block is hung inside the toilet cistern and is designed to retain its effectiveness for up to 6 weeks (or 550-600 flushes).

For college toilets, the daily disinfection procedure involved the addition of 20ml (Scott 1981) of approximately 8-10% W/V available chlorine hypochlorite solution (brand name 'Domestos', Lever Bros., Port Sunlight) to the toilet. For hospital toilets the daily disinfection procedure involved the application of a quaternary ammonium/detergent disinfectant product (brand name 'Bioscan', Hospital General Supplies, Reigate, Surrey). This was the product normally used in the hospital.

8.2.2 Wastetraps

The continuous release disinfectant system for the wastetrap was also based on the solid compound trichloroisocyanuric acid. A total of 3.5 g of coarse grade trichloroisocyanuric acid pellets (brand name 'Fi-Clor 91', Chlor-Chem Ltd., Harston, Cambridge) were packed into a stick-like plastic mesh container in the laboratory. This container was inserted through the plug-hole grid and suspended downwards into the wastetrap.

8.2.3 Neutralization

Trials indicated that the incorporation of neutralizers into solid media for contact sampling of disinfected surfaces was not necessary.

A similar conclusion was reached by the author in a previous field study (Scott 1981). Water samples (10ml) from toilets and wastetraps treated with CRD chlorine disinfectants were inactivated by the addition of 0.1 molar sodium thiosulphate (0.2ml).

Laboratory studies (Appendix 3) indicated that this concentration successfully neutralized chlorine levels of up to 10 ppm without causing inhibition of bacterial growth.

8.2.4 Determination of chlorine levels

At each time of sampling, free chlorine levels in toilet water and wastetrap water samples treated with trichloroisocyanuric acid (continous release disinfectant system) were estimated by a rapid visual colorimetric analysis using a Lovibond 2000 comparator and N,N-diethyl-p-phenylenediamine tablets (DPD) (Tintometer Ltd., Salisbury). This method is based on the DPD colorimetric assay method described by Taras et al (1971) and gives an upper reading of 4 ppm free chlorine.

8.2.5 Test Programme

8.2.5.1 Toilets

During an initial two week period, the daily disinfection (DD) procedure was carried out at both hospital and college toilets. At the end of this period the continous release disinfectant (CRD) blocks were installed and sampling was started again after 2 days and continued for 6 weeks. During this period domestic staff reverted to their "normal" cleaning routine which, for hospital toilets, involved the additional use of the quaternary ammonium/detergent disinfectant product. Throughout the test period, daily cleaning and disinfection, as specified, was carried out by domestic staff early in the morning before daily sampling.

For the first 5 weeks, sampling was carried out twice a day (a.m. and p.m.) on 2 days per week. For the final 3 weeks of the test period (the last 3 weeks of CRD testing), sampling was carried out once a day (p.m.) on 2 days per week.

For the first 4 weeks of the study, all sites (see page 161) were included in the sampling programme whilst for the final 4 weeks of the study (the last 4 weeks of CRD testing), sampling was limited to the bowl water and toilet bowl surface only.

8.2.5.2 Wastetraps

As in the previous Chapter (page 163), sampling was carried out once a day (a.m.), on 4 days per week, over 2 weeks. The continuous release disinfectant stick was installed 3 days before sampling was started. The CRD stick was replenished before the second week of sampling.

8.3 Results

8.3.1 Toilets

8.3.1.1 Colony Counts

Table 35 shows the cumulative frequency of occurrence of colony counts of 12,000, 1,000, 600, 100, 10, 1 or more per ml of bowl water expressed as a percentage of samples taken during the 2 week period of daily disinfectant testing (DD) and the first two weeks of continuous release disinfectant testing (CRD). Control results from Chapter 7 page 165 are also given to enable comparison. In college toilets, 10% of bowl water samples had counts of more than 600/ml in the hypochlorite DD trial and 0% had counts of more than

Table 35 Colony counts in toilet bowl water samples from college and hospital toilets treated with disinfectant over a 2 week period of sampling

Cumulative frequency of occurrence as a percentage of samples taken

Colony counts per ml of toilet water	College			Hospital		
	Control	*DD	*CRD	Control	*DD	*CRD
48,000 or more	-	-	-	0	12	0
12,000 or more	-	-	-	43	32	0
1,000 or more	22	4	0	79	68	2
600 or more	27	10	0	83	73	2
100 or more	49	37	2	95	83	2
10 or more	96	79	4	95	85	5
1 or more	100	94	74	95	85	19
Total number of samples	45	48	47	42	41	43

*DD - daily disinfect *CRD - continuous release disinfectant

600/ml in the CRD trial, compared with 27% in control samples. Although counts of zero were recorded in 26% of the CRD treated toilets, very few zero counts were recorded in the DD treated toilets and none in control samples.

In the hospital trial, 32% of samples in the quaternary ammonium DD treated toilets had counts of 12,000 or more per ml compared with 43% of control samples. CRD treated toilets showed a substantial reduction in colony counts with only one sample (2%) giving a count of 1,000 or more per ml and no growth recorded in 81% of samples. Highest counts were actually recorded during DD testing with 12% of counts greater than 48,000 per ml.

Tables 36 and 37 show results obtained from sites other than bowl water over the same period for college and hospital toilets respectively. For both hospital and college toilets there was a substantial reduction in the occurrence of colony counts of 100 and 10 or more per sample area for the toilet bowl, rim and seat surfaces in CRD treated toilets when compared with control and DD treated toilets. In college, but not hospital toilets, reductions were also achieved in DD toilets compared with control results at these sites. Results for toilet handle, floor and air samples indicate that none of the disinfection procedures applied to the toilets had any significant effect on contamination of surrounding areas in college or hospital toilets, although for floor and air samples there was some reductions in the occurrence of counts greater than 100.

Table 36 Colony counts at individual sites (other than water) from hospital toilets treated with disinfectant over 2 week period of sampling

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken								
	Bowl surface			Rim			Seat		
	Control	*DD	*CRD	Control	*DD	*CRD	Control	*DD	*CRD
100 or more	47.5	50	8	36	44	0	22	21	10
10 or more	90	95	64	44	59	7	67	71	76
1 or more	97.5	100	96	79	100	31	95	91	98
Total number of samples	41	39	41	40	38	40	41	38	42

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken								
	Handle			Floor			Air		
	Control	*DD	*CRD	Control	*DD	*CRD	Control	*DD	*CRD
100 or more	22	29	20	88	100	83	16	0	26
10 or more	78	76	64	98	100	100	100	100	100
1 or more	100	93	72	98	100	100	100	100	100
Total number of samples	42	41	26	42	38	41	19	12	20

*DD - daily disinfectant *CRD - continuous release disinfectant

Table 37 Colony counts at individual sites (other than water) from college toilets treated with disinfectant over a 2 week period of sampling

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken								
	Bowl surface			Rim			Seat		
	Control	*DD	*CRD	Control	*DD	*CRD	Control	*DD	*CRD
100 or more	54	40	9	46	8	7	26	17	7
10 or more	93	74	55	76	56	17	85	83	72
1 or more	98	87	98	89	69	43	100	96	98
Total number of samples	46	48	47	45	38	48	46	48	47

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken								
	Handle			Floor			Air		
	Control	*DD	*CRD	Control	*DD	*CRD	Control	*DD	*CRD
100 or more	6	10	11	92	60	34	78	55	57
10 or more	42	82	38	100	96	100	96	100	100
1 ore more	86	91	78	100	98	100	100	100	100
Total number of samples	37	30	39	40	48	47	23	22	23

*DD - daily disinfectant *CRD - continuous release disinfectant

Colony counts in CRD treated toilet water and bowl surface samples were monitored over a total of 6 weeks, this being the recommended life of the CRD blocks, and results are given in Table 38 . Comparison of Table 38 with Tables 35 , 36 and 37 , indicates that counts were lowest during the third and fourth weeks of monitoring but, although some increase in contamination was observed in weeks 5/6, occurrence of high counts (100 or more) remained small in comparison with toilets which were disinfected on a daily basis. Analysis of data from individual toilets indicated that loss of activity for the CRD system in the final 2 weeks was confined to 4 heavily used toilets.

8.3.1.2 Bacterial species

Although monitoring of colony counts gives an important measure of the efficiency of disinfection procedures, of equal importance is the elmination of species which may constitute an infection hazard.

Tables 39 & 40 shows the effect of disinfection procedures on the incidence of individual bacterial species and groups of species in toilet and surrounding sites. Results indicate that installation of CRD blocks produced a significant reduction in the occurrence of E.coli and other enterococci, Ps. aeruginosa and other 'pseudomonads' in the toilet (bowl water, bowl surface and rim sites) compared with control results. Results for DD treated toilets also indicate some reductions in the occurrence of enterobacteria (but not E.coli) and Ps. aeruginosa at those sites. By comparison with the toilet itself, the overall occurrence of E. coli and other enterobacteria

Table 38. Colony counts in continuous release disinfectant (CRD) treated water and bowl surface samples over weeks 3-6 in college and hospital

Colony counts (per ml. or per 25cm^2)	Cumulative frequency of occurrence as a percentage of samples taken							
	College				Hospital			
	Toilet water weeks		Bowl surface weeks		Toilet water weeks		Bowl surface weeks	
	3-4	5-6	3-4	5-6	3-4	5-6	3-4	5-6
100 or more	0	4	6	30	0	14	11	18
10 or more	6	21	45	70	0	23	34	55
1 or more	57	83	85	90	40	77	96	95
Total number of samples	35	24	34	23	35	22	29	22

Table 39 Frequency of occurrence of bacterial species at toilet and surrounding sites from hospital toilets treated with disinfectant over a 2 week period of sampling

Frequency of occurrence	Hospital					
		*WBR			*SHAF	
	Control	*DD	*CRD	Control	*DD	*CRD
E. coli	8	20	2	9	4	3
Other enterobacteria	50	40	6	14	15	19
Enterococci	1	1	3	11	9	10
Other streptococci	1	2	5	24	26	23
Ps. aeruginosa	58	22	6	8	2	0
Other 'pseudomonads'	28	41	6	15	4	3
Staph. aureus	0	0	0	0	0	0
Micrococci	39	42	40	134	112	117
Gram positive bacilli	39	50	44	84	66	63
Total number of samples	123	118	129	144	129	129

*WBR - water, bowl surface and rim sites (toilet)

*SHAF - seat, handle, floor and air sites (surrounding)

*DD - daily disinfectant

*CRD - continuous release disinfectant

Table 40 Frequency of occurrence of bacterial species at toilet and surrounding sites from college toilets treated with disinfectant over a 2 week period of sampling

Frequency of occurrence	College *WBR			College *SHAF		
	Control	*DD	*CRD	Control	*DD	*CRD
E. coli	33	55	3	8	8	2
Other enterobacteria	54	4	0	2	4	0
Enterococci	11	10	0	20	18	2
Other streptococci	20	20	11	28	37	51
Ps. aeruginosa	11	0	0	0	0	0
Other 'pseudomonads'	39	13	28	54	33	42
Staph. aureus	2	0	0	0	0	0
Micrococci	92	75	47	131	133	147
Gram positive bacilli	83	64	70	83	87	87
Total number of samples	136	134	142	146	148	156

*WBR - water, bowl surface and rim sites (toilet)

*SHAF - seat, handle, floor and air sites (surrounding)

*DD - daily disinfectant

*CRD - continuous release disinfectant

and Ps. aeruginosa at sites surrounding the toilet (seat, handle, floor and air) was relatively infrequent so that reductions in contamination were more difficult to assess. Nevertheless, it was found that installation of CRD blocks produced some reduction in the occurrence of E.coli and Ps. aeruginosa at these sites. Reductions were also observed in the occurrence of enterococci and other 'pseudomonads' at these sites during CRD treatment. Daily disinfection of hospital toilets with quaternary ammonium disinfectants also produced a reduction in the occurrence of E. coli, Ps. aeruginosa and other 'pseudomonads' at surrounding sites compared with control results, but disinfection of college toilets with hypochlorite had little or no effect.

8.3.2 Chlorine levels

Average levels of free available chlorine (ppm) in college and hospital CRD treated toilets for each week of testing are given in Tables 41 and 42 respectively. Although the CRD system is designed to release 4 ppm available chlorine into the bowl water with each flush, it can be seen that chlorine levels less than 4 ppm were frequently recorded. In this investigation, average chlorine levels ranged from 0 to 4 ppm. This is probably due to the fact that chlorine monitoring was carried out at the time of bacteriological sampling and not necessarily immediately following flushing when levels would be expected to be at their highest. Although some reduction in average chlorine levels occurred over the final 3 weeks of testing, the reduction was marginal and analysis

Table 41 Average chlorine levels (p.p.m.) in hospital toilets over 6 weeks

Week	Average levels of free available chlorine (p.p.m.)					
	Hospital toilets (x 6)					
	1	2	3	4	5	6
1	3.1	0.15	1.6	4.0	2.4	2.7
2	2.9	1.8	1.3	3.4	2.0	1.75
3	3.6	0.1	1.2	3.5	1.7	1.2
4	2.0	1.5	0.3	1.5	1.25	0.2
5	1.75	1.5	0.6	0	0.4	0
6	0.5	2.5	2.5	0	0	0

Table 42 Average chlorine levels (p.p.m) in college toilets over 6 weeks

Week	Average levels of free available chlorine (p.p.m.) College toilets (x 6)					
	1	2	3	4	5	6
1	2	1.6	3	4	2.3	1
2	1.6	2.7	4	2.75	2.25	2.25
3	1.4	2.5	2.5	1.4	1.0	0.4
4	1.75	2.0	2.75	0.3	1.75	0
5	1.25	2.75	4.0	0	0.3	0.3
6	1.35	2.0	4.0	0	0.2	0

of data from individual toilets indicated that it was confined to the 4 most heavily used toilets. It was at thosesame 4 toilets that loss of activity was observed (page 191). The remaining 8 CRD blocks continued to be effective over a 5-6 week period.

8.3.2 Wastetraps

8.3.2.1 Colony counts

Colony counts from CRD treated wastetraps and from sink surfaces sampled in conjunction with treated wastetraps are shown in Tables 43and 44 respectively. Control results from Chapter 7 page 171 are also given.

The CRD stick was very effective in the wastetrap with zero counts recorded in all 8 samples compared with counts of greater than 10^4 organisms/ml in all control samples. However, despite the effectiveness of the CRD stick in the wastetrap, the results in Table 44 do not indicate any concomitant trend to reductions in colony counts at the sink surface, although more data would be required to confirm these results.

8.3.2.2 Bacterial species

Gram negative bacilli isolated from CRD treated wastetraps and sink surfaces sampled in conjunction with treated wastetraps are shown in Tables 45 and 46 respectively. Installation of the CRD stick resulted in the total elimination of Gram negative bacilli from the wastetrap. However, even in the absence of Gram negative bacilli from the wastetrap, the range and frequency of occurrence of Gram negative bacilli isolated from the sink surface was little affected.

Table 43 Colony counts of Gram negative bacilli/ml of control and disinfected wastetrap liquid sampled over a 2 week period

Colony counts per ml of wastetrap water	
Control	CRD
1.3×10^5	0
3.9×10^6	0
6×10^5	0
1.6×10^5	0
1.2×10^4	0
7.75×10^6	0
5.6×10^5	0
2.5×10^6	0
Total no. of samples 8	8

Table 44 Colony counts of Gram. negative bacilli/25cm^2 recovered by contact from a sink surface sampled in conjunction with control and disinfected wastetrap liquid over a 2 week period

C.F.U's/25cm^2 sink surface	
Control	CRD
TNTC	TNTC
TNTC	TNTC
TNTC	TNTC
TNTC	TNTC
33	TNTC
TNTC	105
TNTC	82
TNTC	TNTC
Total number of samples	8	8

Table 45 Frequency of occurrence of Gram negative bacilli isolated from control and disinfected wastetrap water over a 2 week period of sampling

	Frequency of occurrence	
	Control	CRD
E. coli	1	0
Ent. cloacae	3	0
C. freundii	4	0
Ps.fluorescens qp.	2	0
Pseudomonas spp (poss. Ps.alcaligenes)	1	0
Total number of samples	8	8

Table 46 Frequency of occurrence of Gram negative bacilli isolated from a sink surface sampled in conjunction with control and disinfected wastetrap liquid over a 2 week period of sampling

	Frequency of occurrence	
	Control	CRD
Enc. cloacae	4	3
C. freundii	3	6
Ps.fluorescens gp	0	1
Pseudomonas spp.	1	0
(poss. Ps. acaligenes)	1	0
Total number of samples	8	8

8.3.2.3 Chlorine levels

The recorded levels of free available chlorine (ppm) in CRD treated wastetrap water over 2 weeks are given in Table 47 . A fresh CRD stick was installed for each of the 2 weeks of testing and each stick remained effective throughout the week. Chlorine levels did not appear to fall off towards the end of the week and although chlorine was undetectable on one occasion (day 3, week 1), this sample was taken following operation of a washing machine, with an outlet plumbed via the sink wastetrap, causing massive 'flushing' through the wastetrap.

Table 47 Free available chlorine levels (p.p.m.) in disinfected wastetrap water sampled over 2 weeks

Sample days		Free available chlorine (p.p.m.)
week 1	(1	4.0
	(2	0.4
	(3	0*
	(4	1.5
week 2	(7	0.2
	(8	4.0
	(9	1.5
	(10	3.0

*Day 3 sample followed operation of washing machine with water outlet plumbed via sink wastetrap

8.4 Discussion

Tests with disinfectant treatments on toilets described in this chapter indicated that daily use of hypochlorite or quaternary ammonium products produced some reduction in contamination in the toilet itself (water, bowl surface and rim), but the effects were fairly limited and generally indicated the inadequacy of daily disinfection of toilets in maintaining any real reduction in microbial contamination compared with those associated with normal cleaning (control results chapter 7 page 164). Sampling of toilets and toilet areas following installation of CRD blocks in hospital and college toilets indicated that these systems produced substantial and sustained reductions in microbial contamination; during the first 2 week sampling period 96% of toilet water samples had counts of less than 10 organisms per ml and 66% of bowl and rim samples had counts less than 10 organisms per sample area. The CRD system appeared to be particularly effective in eliminating Gram negative organisms from water, bowl surface and rim sites; out of a total of 266 samples, not more than 6% were found to be contaminated with a Gram negative organism compared with 56% of DD samples. The fact that the CRD blocks produced a reduction in counts and occurrence of Gram negatives on toilet seat surfaces is of interest in view of the possible role of these surfaces in the spread of infection outbreaks such as sonne dysentery (Hutchinson 1956).

By contrast however the reduction in contamination at sites such as toilet handles, floor and air samples associated with toilet disinfection was relatively small although, for example, whereas 8 isolates of Ps. aeruginosa were obtained from these sites when hospital toilets were subjected to daily cleaning only (control results page 169), no further isolates were obtained following installation of the CRD block. The fairly limited effects at these sites, compared with the substantial reductions in the toilet water, suggests that, for most species, particularly in hospital toilet areas, a substantial proportion of the contamination arising in areas surrounding the toilet occurs by direct shedding or transfer from the patient rather than via aerosol production from the toilet as described by other authors (Darlow & Bale 1959, Bound & Atkinson 1966 and Gerba et al 1975). There are indications however that Ps. aeruginosa isolated from toilet surfaces may arise largely from contamination in toilet water although more extensive studies would be required to confirm this.

The CRD stick system in the wastetrap produced better results than those observed in a previous study (Scott 1981) in which wastetraps were disinfected daily with a single application of sodium hypochlorite on 3 consecutive days. In the earlier study, even though reduced colony counts were recorded from wastetrap water samples one hour after treatment, subsequent samples taken 6 hours after disinfection indicated that colony counts had returned to their pre-treatment levels.

Comparing the results of a single application of disinfectant (Scott 1981) with the current study indicates that a CRD system will maintain reduced levels of contamination in the wastetrap. Similar results were achieved by Kohn (1970) using heat as a means of disinfection. By contrast, Thomas et al (1972) found a single daily application of neat disinfectant (Hycolin) to be effective.

CHAPTER 9

FIELD STUDIES ON THE RELATIONSHIP BETWEEN BACTERIAL CONTAMINATION OF FOOD PREPARATION SURFACES AND CLEANING CLOTHS

9.1 Introduction

Literature concerned with the problems associated with the use of contaminated cloths has been reviewed in the introduction to a previous chapter (chapter 4). The potential for the survival and transfer of contamination from cloths to worksurfaces has been established by controlled laboratory experiments described in this thesis (chapter 4). As a result of further laboratory experiments some of the problems involved in bringing about effective decontamination of re-usable cloths have been explored (chapter 5).

This chapter sets out to establish the levels of contamination associated with the use of cloths in a catering establishment under normal conditions and to evaluate a disinfectant - impregnated cloth for use on food preparation and related surfaces under "in-use" conditions. The kitchen used in this study was that at Queen Elizabeth College (Q.E.C.), London University. In this kitchen, 200-300 lunch-time covers were prepared daily and catering for additional functions was also carried out.

9.2 Methods

9.21 Sampling sites and methods of sampling worksurfaces and cloths.

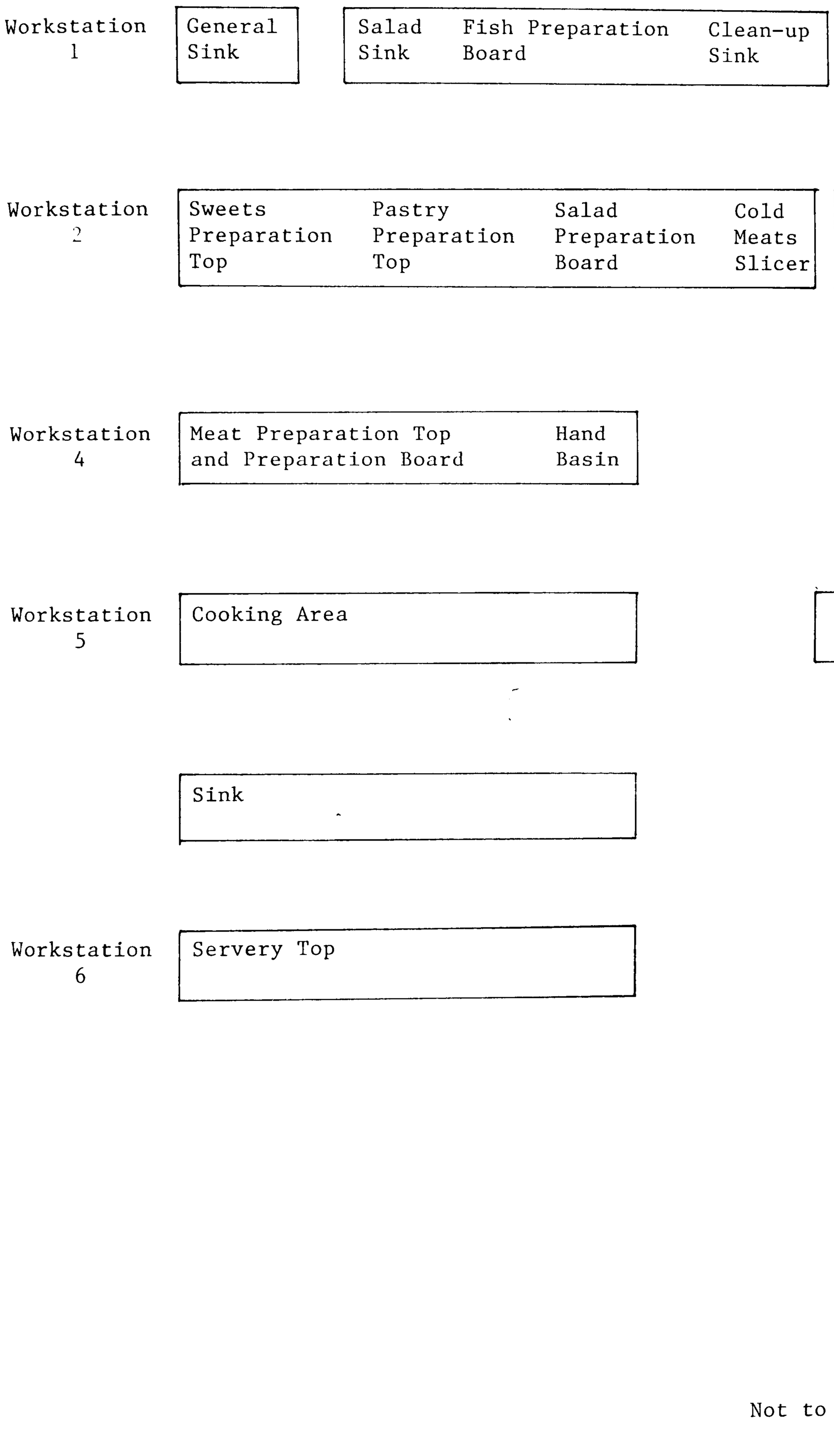
The kitchen at QEC was typically arranged to keep different food preparation procedures separate from one another. The kitchen consisted of 6 workstations plus a refrigeration area as shown in Figure 5 .

A total of 12 surfaces were monitored within the kitchen and a separate cleaning cloth was assigned to each of the 6 workstations and the refrigeration area. Surfaces and cleaning cloths monitored in this study are listed in Table 48 .

Sampling procedures were based on a previously published method (Scott et al 1981). Cloth and work surfaces were sampled by placing nutrient agar contact plates (with or without neutralizers as appropriate, Chapter 2 page 67) in contact for 10s. Serum-coated swabs (Exogen Ltd) pre-moistened with one quarter strength Ringer's solution were also used to sample areas of worksurface and cloth approximately 50 cm^2 adjacent to the contact area. The swabs were returned immediately to their plastic containers.

Samples were returned to the laboratory in an insulated cool box within 1h of collection. Swabs were streaked onto MacConkey agar (Tissue Culture Services Ltd, Slough). All plates and slides were incubated aerobically at 37°C for 24h.

Figure 5 Layout of QEC Kitchen



Not to scale

Table 48 Surfaces and cleaning cloths monitored in the QEC kitchen

Surface	Cleaning Cloth
Sandwich Preparation Top	Sandwich Top Cloth
Sweets Preparation Top Pastry Preparation Top Salad Preparation Board Cold Meat Slicer	Sweets Top Etc Cloth
Salad Sink Fish Preparation Board Clean-up Sink	Sink Cloth
Meat Preparation Top Meat Preparation Board	Meat Top Cloth
Fridge Shelf	Fridge Cloth
Servery Top	Servery Top Cloth

9.2.2 Enumeration and identification of bacteria

Total viable counts from hard surfaces and cloths were made from colony counts on contact plates. For identification of contaminants, colony morphology and Gram strain reactions were determined for all isolates from contact plates and McConkey agar plates. Enterobacteria and Pseudomonads were further identified using the API 20E System (Chapter 2 page 60).

9.2.3 Cloths

Two cleaning systems involving cloths were used in this study.

The control system involved disposable 'J-cloth' type cloths (Chapter 2 page 64) together with a solution of anionic detergent (supplied by QEC kitchens) which were used to wipe down surfaces (detergent cloth). This was the usual cleaning system employed by the kitchen staff.

The second system involved the use of a patented non-woven viscose/cellulose fabric cloth onto which is bonded a blend of cationic disinfectants comprising a quaternary ammonium compound, bisguanide and EDTA (quaternary ammonium or QA cloths). Blue stripes are bonded to a portion of the disinfectant and loss of activity is indicated by the disappearance of these stripes. These cloths were marketed to the catering industry and supplied by Wipex Products Ltd (London).

9.2.4 Sampling programme

A total of 6 new cloths were incorporated at their appropriate sites at the start of kitchen activities on day 1 of each test.

Staff were responsible for the maintenance and use of cloths at the appropriate sites. Before the start of the trial, staff were instructed in the correct system for the use of the disinfectant impregnated cloth. This involved removal of any organic soil using a paper towel after which the surface was wiped with the damp disinfectant cloth. The procedure for the use of control cloths was based upon normal practice in the kitchen and involved the removal of organic soil using a paper towel after which the surface was wiped with the damp detergent cloth.

Cloths (except fridge and sink cloths) were used at the appropriate sites for all activities where cloths were required for the duration of the test (2 days). Sink and fridge cloths were used to wipe surfaces only once a day at the time of sampling.

Sampling of surfaces and cloths was carried out at 10.30 am by which time most of the daily preparation activities were near completion. Surfaces and cloths were sampled with contact plates and swabs (post preparation) and staff were then requested to "clean" the test surfaces with the appropriate moist detergent cloths or Q.A. cloth. All surfaces and cloths were resampled (post cleaning) at an interval of 5 minutes following "cleaning". This sampling programme was repeated over 2 days.

9.3 Results

Colony counts per 25 cm^2 for surfaces and cloths are recorded in Tables 49 to 51 .

Post-preparation samples on day 1 of the detergent study (Table 49) indicate that some cloths (2/6, sweets top and servery cloth) became heavily contaminated (greater than 100 colonies per 25 cm^2) within a few hours of use. In the following 24 hours, 5 out of 6 of the detergent cloths gave counts of greater than 100/25 cm^2 (Table 49).

Post-preparation samples on day 1 also indicate that in general surfaces were heavily contaminated. A total of 7/12 surfaces in the detergent study and 6/12 surfaces in the Q.A. study gave counts of greater than 100/25 cm^2.

Following "cleaning" (ie post-cleaning) cloths and surfaces in the detergent study were found to be more heavily contaminated than immediately prior to cleaning on day 1. All 6 of the detergent cloths and 10/12 surfaces recorded counts of greater than 100/25 cm^2. The results indicate that in some cases, (eg servery) contamination was transferred from the cloths to the surface whilst in other cases (eg sink) transfer occurred from surface to cloth.

The same pattern of increases in contamination of surfaces and cloths following "cleaning" was observed on day 2. In only one instance over 2 days of sampling was a lower count recorded from a surface following wiping with a detergent cloth (sandwich top, day 2)

Table 49 Counts on detergent cloths and surfaces post preparation and 5 mins. post cleaning on day 1

	Counts/25 cm^2				
	CLOTHS			SURFACES	
	Post Prepn.	Post Clean		Post Prepn.	Post Clean
Sandwich Top	42	120	Sandwich Top	55	70
Sweets Top	TNTC	TNTC	Sweets Top	TNTC	TNTC
			Pastry Top	TNTC	TNTC
			Salad Top	TNTC	TNTC
			Meat Slicer	120	TNTC
Sink	4	TNTC	Salad Sink	TNTC	TNTC
			Fish Board	TNTC	TNTC
			Clean-up Sink	TNTC	TNTC
Meat Top	0	TNTC	Meat Top	30	TNTC
			Meat Board	27	120
Fridge	12	TNTC	Fridge Shelf	13	86
Servery	TNTC	TNTC	Servery Top	7	TNTC
Proportion of counts of <10	2/6 (33%)	0		1/12 (8·3%)	0
Proportion of counts of >100	2/6 (33%)	6/6 (100%)		7/12 (58%)	10/12 (83%)

Table 50 Counts on detergent cloths and surfaces post preparation and 5 mins. post cleaning on day 2

Counts/25 cm^2					
CLOTHS			SURFACES		
	Post Prepn.	Post Clean		Post Prepn.	Post Clean
Sandwich Top	104	112	Sandwich Top	TNTC	110
Sweets Top	TNTC	TNTC	Sweets Top	TNTC	TNTC
			Pastry Top	TNTC	TNTC
			Salad Top	TNTC	TNTC
			Meat Slicer	TNTC	TNTC
Sink	28	TNTC	Salad Sink	TNTC	TNTC
			Fish Board	25	44
			Clean-up Sink	TNTC	TNTC
Meat Top	TNTC	TNTC	Meat Top	TNTC	TNTC
			Meat Board	TNTC	TNTC
Fridge	104	107	Fridge Shelf	44	TNTC
Servery	TNTC	TNTC	Servery Top	TNTC	TNTC
Proportion of counts of <10	0	0		0	0
Proportion of counts of >100	5/6 (83%)	6/6 (100%)		10/12 (83%)	11/12 (92%)

Table 51 Counts on Q.A. coths and surfaces post preparation and 5 mins. post cleaning on day 1

Counts/25 cm^2					
CLOTHS			SURFACES		
	Post Prepn.	Post Clean		Post Prepn.	Post Clean
Sandwich Top	0	0	Sandwich Top	20	0
Sweets Top	3	1	Sweets Top	73	57
			Pastry Top	1	0
			Salad Top	TNTC	88
			Meat Slicer	100	41
Sink	0	1	Salad Sink	TNTC	0
			Fish Board	TNTC	31
			Clean-up Sink	TNTC	8
Meat Top	0	0	Meat Top	0	0
			Meat Board	2	0
Fridge	0	0	Fridge Shelf	TNTC	0
Servery	0	0	Servery Top	8	0
Proportion of counts of <10	6/6 (100%)	6/6 (100%)		4/12 (33%)	8/12 (66%)
Proportion of counts of >100	0/6 (0%)	0/6 (0%)		6/12 (50%)	0/12 (0%)

Table 52 Counts on Q.A. cloths and surfaces post preparation and 5 mins. post cleaning on day 2

	Counts/25 cm^2				
	CLOTHS			SURFACES	
	Post Prepn.	Post Clean		Post Prepn.	Post Clean
Sandwich Top	0	0	Sandwich Top	80	0
Sweets Top	14	17	Sweets Top	100	15
			Pastry Top	100	30
			Salad Top	TNTC	12
			Meat Slicer	1	60
Sink	0	0	Salad Sink	TNTC	0
			Fish Board	TNTC	3
			Clean-up Sink	0	0
Meat Top	0	14	Meat Top	2	0
			Meat Board	200	100
Fridge	0	0	Fridge Shelf	0	0
Servery	200	TNTC	Servery Top	TNTC	TNTC
Proportion of counts of <10	4/6 (66%)	3/6 (50%)		4/12 (33%)	6/12 (50%)
Proportion of counts of >100	1/6 (17%)	1/6 (17%)		7/12 (58%)	2/12 (17%)

and in this example, the count remained greater than 100/25 cm^2 (Table 50)

By contrast, in the Q.A. study, only 2 out of 24 surface samples showed either an increase in count following "cleaning" (meat slicer, day 2) or a count recorded as TNTC in both pre and post clean sampling (servery top, day 2). In 22 out of the 24 surface samples over 2 days, the count was reduced at post-clean and in 5 of those samples, the count was reduced from uncountable to less than 10/25 cm^2. The cloth counts remained low with 19 out of the 24 post preparation and post clean samples over 2 days recording counts of 10 or less/25 cm^2 (Tables 51 and 52)

For the purposes of this study, the frequency of occurrence of Gram negative species on cloths and surfaces comprising enterobacteria and pseudomonads was investigated. These organisms originate from raw food and may therefore be regarded as indicators of poor hygiene. In this study the following organisms were isolated and identified:

Enterobacteria

<u>K. pneumoniae</u>

<u>Ent. cloacae</u>

Pseudomonads

Pseudomonas spp

Ps. fluorescens gp

<u>Acinetobacter calcoaceticus</u>

Achromobacter spp

The frequency of occurrence of these groups of species on surfaces and cloths are shown in Table 53 and Table 54 respectively. The results indicate that

Table 53 The occurrence of enterobacteria and pseudomonads on surfaces at all pre and post clean samples over 2 days

BACTERIAL GROUP	Occurrence at all surfaces in the DETERGENT CLOTH System (24 samples)		Q.A. CLOTH System (24 samples)	
	Post Preparation	Post Clean	Post Preparation	Post Clean
Enterobacteria	18	21	11	6
Pseudomonads	5	9	3	1

Table 54 The occurrence of enterobacteria and pseudomonads on cloths at all pre and post clean samples over 2 days

BACTERIAL GROUP	Occurence on all cloths in the			
	DETERGENT CLOTH System (12 samples)		Q.A. CLOTH System (12 samples)	
	Post Preparation	Post Clean	Post Preparation	Post Clean
Enterobacteria	8	10	1	2
Pseudomonads	3	4	0	0

the incidence of these organisms following "cleaning" with detergent only was little different from that prior to cleaning.

In the Q.A. System there was found to be little difference in the incidence of enterobacteria and pseudomonad contamination of cloths before as compared with after cleaning but some reduction in the frequency of contamination of surfaces with these species was observed. Overall, there was a reduction in the total incidence of enterobacteria and pseudomonad contamination in the Q.A. cloth study as compared with the detergent cloth study.

Discussion

Bacterial counts from samples taken from cloths and surfaces used in a catering environment confirm and extend the findings of other workers (Davis et al 1968, Tebbutt 1986, Mendes et al 1978) and indicate that both cloths and surfaces become heavily contaminated during food preparation activities.

Organisms recovered included species of enterobacteria isolated from upto 87% of food contact surfaces and upt 83% of cloth samples indicating potential deficiencies in kitchen hygiene. Some cloths were heavily contaminated after only 3 hours in use.

Whereas initial sampling of detergent cloths on day 1 showed only 2 cloths were heavily contaminated, by day 2 all of the cloths were then heavily contaminated. This indicates (as found in laboratory studies described in chapter 4) the survival and possibly also proliferation of bacteria in damp cloths during the intervening storage period and probably accounts for the general overall increase in contamination associated with both cloths and surfaces in the detergent study on day 2 compared with day 1.

The results of these studies show that,where detergent cloths were used to clean surfaces after food preparation, there was an increase in contamination. In some cases, this appeared to result from transfer from cloths to surfaces and in others from surfaces to cloths. The overall result was that at post-cleaning on day 1, 16 out of 18 (89%), and on day 2, 17 out of 18 (94%) of all the surface and cloth samples

were heavily contaminated and that organisms indicative of poor hygiene were frequently isolated. These results are in agreement with those of other workers (Davis et al 1968, Gilbert 1969, Tebbutt 1986).

It is suggested that although some increase in contamination on surfaces may result from break-up and redistribution of cell aggregates, some of the increase must also be due to the spread of contamination via cloths.

The results indicate the potential shortcoming associated with the use of reusable cloths in conjunction with a detergent cleaning system. Results for sink and fridge show that even where a relatively clean cloth is used in combination with a detergent for "cleaning", surfaces are not decontaminated.

Results with Q.A. cloths indicate that the use of disinfectant impregnated cloths produces a significant improvement in the hygiene of food preparation surfaces such that at post cleaning on day 1, none (0%) and on day 2, only 3 out of 18 (16%) of cloths and surfaces were heavily contaminated. There was also a significant reduction in the incidence of enterobacteria and pseudomonads. Even where enterobacteria and pseudomonads did occur on surfaces, their numbers were greatly reduced. The highest colony count for enterobacteria recorded from surfaces in the Q.A. study was 56/25 cm^2 and only 9% of surfaces had count of greater than 20. In comparison, 83% of surfaces in the detergent study had colony counts for enterobacteria of greater than 100/25 cm^2.

Overall, the results of this study illustrate clearly that the use of cloths on food preparation surfaces and without application of disinfectants is associated with a build up of high levels of contamination on these surfaces. The results also show that cleaning these surfaces with detergent and water does not achieve decontamination but merely transfers organisms onto the cloth to be spread to other areas of surface. The results suggest that adequate decontamination procedures are required to prevent transfer of contamination wherever cloths are applied to food preparation surfaces. The results indicate that disinfectants can be used to achieve a significant reduction in contamination of food preparation surfaces. In this investigation, a self-disinfecting cloth system was chosen to overcome some of the problems of user compliance. Although this system produced a substantial improvement in the hygiene of food preparation surfaces, significant contamination was still recorded on a number of surfaces after cleaning. Further, observations made during this study and also reported by Babb et al (1981) indicate that the cloths are quite frequently misused in a way which causes neutralization of the disinfectant.

This study particularly emphasises the hazards associated with re-usable cloths which may act both as free living reservoirs as well as disseminators of microbial contamination if not correctly handled. It is recognised

that improvements in cloth hygiene could be achieved in a number of ways, either by ensuring that freshly disinfected cloths are used for each operation or by the exclusive use of disposable cloths. Although the use of reusable cloths is actively discouraged in hospitals, our observations suggest that this practice is still widely adopted in certain situations.

CHAPTER 10

GENERAL DISCUSSION

10.1 The potential infection risk associated with microbial contamination in working situations where there is an increased hygiene risk

Bacteriological studies clearly show that potentially pathogenic bacteria can and do originate from all parts of the environment including food, personnel and equipment as well as sites and surfaces in catering and food production, in pharmaceutical manufacture and in hospitals. Whilst many of the constituent parts of the general environment are associated with potentially pathogenic contamination, what is less clear is the frequency with which various sites may be directly or indirectly responsible for producing infection. For any environment, in order to formulate a disinfection/cleaning policy which is cost-effective, an assessment of risk is required.

A consideration of the general environment indicates that the risks associated with a particular site depend on the following two quite separate factors. Firstly, the frequency with which the site may be contaminated with potentially pathogenic species and secondly, the probability of transfer of this contamination to a more sensitive site or surface, or directly to a foodstuff, a product under manufacture or to a patient.

10.1.1. Frequency of contamination

Whilst there is no comprehensive data on the hospital environment or the manufacturing environment, limited studies of hospital pharmaceutical production (Baird et al 1976) and studies of the catering environment (Mendes et al 1978) and the domestic environment

(Scott et al 1982) reveal information which is considered of value in assessing the frequency of contamination of sites with potentially pathogenic species. As discussed in the general introduction to this study, it is suggested that in any general environment there are three groups of sites which are of importance in terms of infection control, namely, reservoir, reservoir/disseminator and contact sites. As indicated in Chapter 1, a number of sites (usually wet or damp) such as sinks and wastetraps can be identified which have the potential to act as permanent or semi-permanent and in some cases, free-living reservoirs of bacteria. Another group comprising all wet cleaning utensils have the potential to act both as reservoirs and because of the nature of their use, as disseminators of contamination. A third group of sites are identified as the contact sites (eg food preparation surfaces, work surfaces, medical equipment, patient care equipment, taps, handles etc.) As discussed in Chapter 1, Page 36 the domestic survey (Scott et al 1982) employed two criteria for assessing the frequency of occurrence of significant contamination at any given site, namely, the frequency of occurrence of enterobacteria as indicators of poor hygiene and the frequency of occurrence of high counts (Table 55). Using these criteria, results from the domestic study indicate that for reservoir sites, the frequency of occurrence of enterobacteria ranges from 30% to 64% and the frequency of occurrence of high counts ranges from 10% to 80%. For reservoir/disseminator sites, the frequency of occurrence of enterobacteria and high counts range

le 55 Frequency of occurrence of contamination at reservoir, reservoir/disseminator and contact sites in the domestic environment

Site assification	Frequency of occurrence of enterobacteria	Frequency of occurrence of high counts
servoir sites	30% - 64%	10% - 80%
servoir/disseminator tes	24% - 30%	28% - 80%
ntact sites	4% - 20%	4% - 40%

00/sample area

f. Scott et al 1982

from 24% to 30% and 28% to 80% respectively. For hand and food contact sites, the frequency of occurrence of enterobacteria and high counts range from 4% to 20% and 4% to 40% respectively.

It must be clearly stated here that although some similarities between the domestic and other general environments must be assumed, it would be erroneous to expect the levels of contamination to be the same. If anything, greater contamination might be expected in other environments such as catering, manufacturing and hospitals. In this study the contamination of hospital and college toilets and catering/food contact surfaces was investigated. The results showed that for hospital and college toilet sites, the frequency of occurrence of high counts ranged from 36% to 95% (Chapter 7 , Page 164) as compared to only 10% for similar sites in the home (Scott et al 1982). For food contact surfaces in the catering environment up to 92% of surfaces were found to have high counts (Chapter 9 , Page 215) compared with 10 to 20% of similar surfaces in the home.

In assessing the contamination of environmental sites it is recognized that the frequency of occurrence of individual species must to a certain extent reflect the ability of these organisms to survive and proliferate at these sites. Whereas there is generally a tendency to assume that potential pathogens have only limited ability to survive and proliferate in the general environment, the relatively high frequency of occurrence of some potential pathogens as illustrated by our domestic and other similar studies suggests that survival and proliferation in such environments is perhaps higher than might be expected.

In this study, experiments with contact, disseminator and reservoir sites which were either artificially or naturally contaminated indicated that although contamination on dry surfaces (in this case a cloth or laminate surface) is rapidly and substantially reduced during even relatively short periods of 1-2 hours drying time, drying action alone is insufficient to guarantee elimination of contamination. All of the organisms studied were found to survive in sufficient numbers to allow their transfer for periods of at least 1 hour whether from clean or dirty surfaces and in many cases, transfer was possible for periods up to 4 hours. Survival over longer periods ranged from 24 hours for Staph. aureus on soiled laminate surfaces (Table 8) and for up to 21 days for Gram negative organisms on naturally contaminated soiled but apparently dry cloths (Table 14). Although where cloths and laminate surfaces were maintained under clean dry conditions the potential for survival was substantially decreased, even clean dry conditions were insufficient to guarantee freedom from contamination, particularly for cloths (Table 9).

Further, whereas organisms may survive for quite long periods on dry surfaces in the absence of moisture, the results show that for certain sites under certain conditions, organisms not only survive but can also proliferate to relatively high numbers within a fairly limited period of time; counts of 10^2 increasing to 10^4 per cm^2 were recorded for naturally contaminated samples of cloths (Table 20) and counts of 10^2 increasing to 10^6/ml for toilet water samples (Table 33).

Although this growth occurred most readily at sites where an ample supply of water was present, results suggest that growth was also observed to a lesser extent on hard laminate surfaces where only a limited amount of moisture was present (Table 8).

10.1.2 Transfer of contamination As discussed earlier, the second factor in assessing the risk associated with a particular site is the likelihood that contamination may be transferred, either directly or indirectly, from the contaminated site to a more sensitive site or surface. As a result of experiments described in this thesis together with other published data, it is possible to begin to assess the risk of transfer for the three site groups of interest, that is, reservoirs, reservoir/disseminators and contact sites.

For reservoirs, the examples of toilets and wastetraps were investigated and the results indicate that under conditions of normal usage the risk of transfer is relatively low.

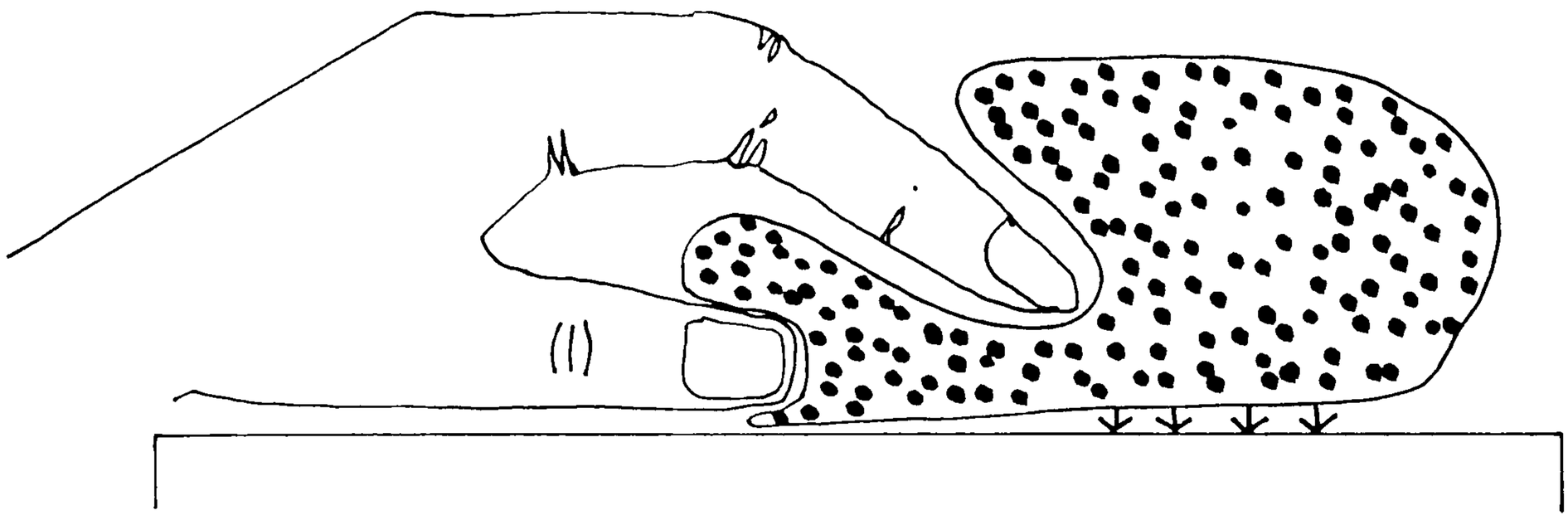
Hospital and college toilets were found to be quite heavily contaminated with colony counts of 600 or more per ml of bowl water in over half of the toilets examined and with Gram negative potential pathogens quite frequently isolated from the toilet and surrounding sites however, whereas successful disinfection procedures reduced contamination in the toilet, effects at surrounding sites were limited. This suggests that much of the contamination arising in areas surrounding the toilet occurs by direct shedding etc., rather than by transfer from the toilet via aerosols or splashing.

Limited investigations into wastetraps also indicate that although wastetraps themselves are heavily contaminated, often with potentially pathogenic organisms, it is difficult to ascertain whether contamination transfer from the wastetrap to surrounding surfaces via a splashback effect occurs to any significant extent. Undoubtedly, wastetrap contamination could be flushed back into the clean washing water if the sink or basin were filled to the overflow connected to the wastetrap.

Overall, the evidence from this investigation, together with evidence from other workers (as discussed in the introduction to Chapter 7, Pages 156 to 157), suggests that under normal usage, the transfer of contamination from toilets via aerosols and from wastetraps via splashback is limited. Whilst both toilets and wastetraps can and do act as reservoirs of contamination, it is suggested that under normal circumstances, the risks of infection transfer associated with toilet and wastetrap contamination are infrequent.

By way of contrast, experiments on cloths indicate that wet cleaning utensils and cloths in particular can act not only as free-living reservoirs but also as disseminators of contamination in the environment. The investigations clearly showed that where contaminated cloths are applied to surfaces, organisms are invariably transferred both to the surface and to the hands of the user. In experiments as summarized in Table 56 using cloths contaminated with as little as 10^5 organisms per cloth, significant numbers of both Staph. aureus

Table 56 Transfer of Bacteria from a contaminated cloth to fingertips and a worksurface



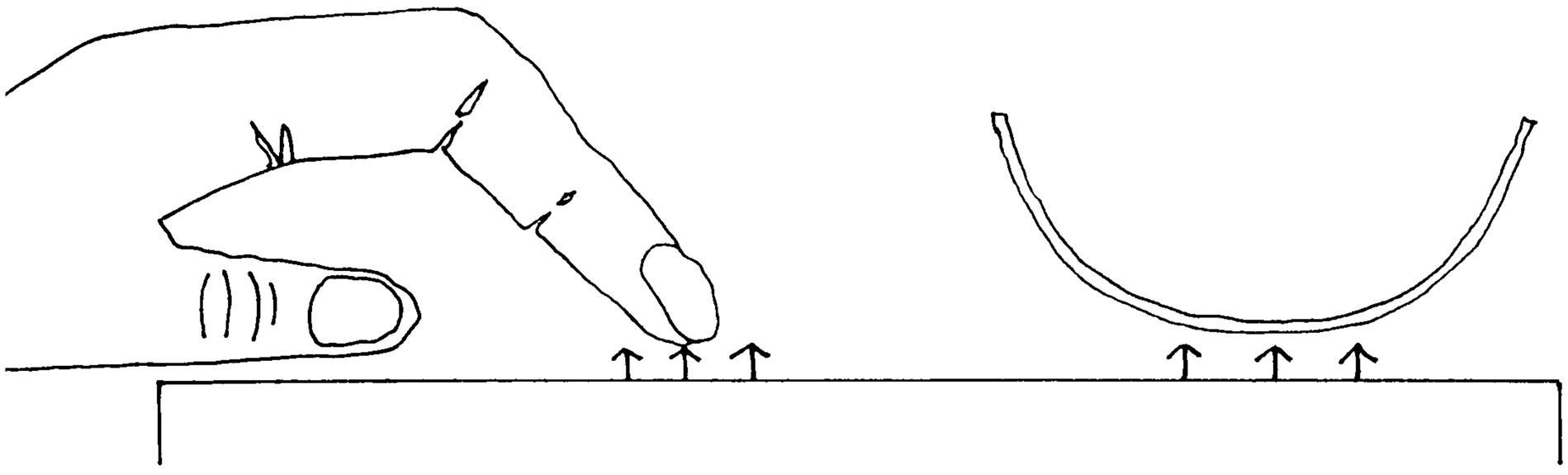
Organism inoculated onto cloth	Number of organisms recovered at 1h. after inoculation from	
	Fingertip	Worksurface 25cm^2
K. aerogenes	34-60	34-39
E. coli	5-7	22-31
Staph. aureus	77-153	27-28

and Gram negative organisms such as E. coli and K. aerogenes were deposited onto the fingers and also transferred to a clean surface. Colony counts from cloths which were returned to the laboratory following 3 days use in the home indicate that in reality cloths are more heavily contaminated (ranging from 10^2 to 10^6 organisms per cm^2 of cloth) than those which were artifically contaminated for experimental use. The risk of transfer of contamination from and via cloths is considerable and such items must therefore be considered as posing a constant risk.

The experiments with worksurfaces, which are classified as a contact site, indicate that, as with cloths, a substantial number of organisms can be tranferred via the hands or via a utensil or patient care item from an apparently dry worksurface contaminated with relatively small numbers of potential pathogens to a potentially more crucial site or surface such as foodstuffs, clean preparation areas, clean equipment/instumentation or perhaps even a patient.

Experiments (summarised in Table 57) using surfaces contaminated with as little at 10^2 organisms/contact area showed that significant numbers between 40 and 99 colonies of Staph. aureus and also Gram negative species such as Salmonella and E. coli could be tranferred via a fingertip or a stainless steel bowl to another surface. Transfer was also demonstrated from surfaces contaminated with Staph. aureus which had been maintained under dry conditions for 24 hours (Table 12). On

Table 57 Transfer of bacteria from a contaminated surface to fingertips or a stainless steel bowl



DRY CONTAMINATED SURFACE approx. 10^2 organisms/contact area

Organism inoculated onto surface	Number of organisms recovered at 1h. after inoculation from:	
	1 Fingertip	Bowl surface
Salmonella spp	55-78	54-95
E. coli	69-62	49-56
Staph. aureus	99-85	40-46

the basis that there is no immediate way of determining whether a particular surface is contaminated or not these contact surfaces (like cloths) must be regarded as a constant risk. Whereas studies on continuous release disinfectants in toilets (Chapter 8) showed that a reduction of contamination in the toilet itself was accompanied by little reduction at surrounding sites, in-use cloth studies (Chapter 9) showed that the use of an effective self-disinfecting cloth was associated with a significant improvement in hygiene at food contact surfaces. This indicates the crucial nature of cloths in the transfer of contamination. The results of these studies also suggest the ease with which surface contamination is transferred to other surfaces, in this case a cloth.

In assessing the potential hazards associated with transfer of contamination with the hands or utensils it must be remembered that the risk of infection depends not only on the type but also the numbers of organisms transferred as discussed in Chapter 1 Page 26 and Chapter 4 Page 108 . It would be pertinent to again note that in a study on experimental aspects of local infections, Marples (1976) stated the inoculum of Staph. aureus required to infect traumatised and occluded skin was actually found to be as little as 20 viable cells. In food poisoning, the numbers of ingestested salmonellae may be as little as 10^2 or even less (Gill et al 1983; Greenwood and Hooper 1983; D'Aoust 1985). These studies clearly show that under certain circumstances, the transfer of a very few organisms can pose a serious hazard.

The results of experiments described in this study, particularly involving cloths and contact surfaces, indicate the potential for transfer of contaminating organisms such as Staph. aureus and Salmonella in numbers greater than the minimum suggested above as likely to pose a hazard. Whereas normal healthy adults and to a varying extent, for example, the hospital patient or neonate will have defence mechanisms which reduce the risk of infection arising from the transfer of contamination, transfer of small numbers of organisms to pharmaceuticals or to foods under conditions which provide a suitable environment for microbial growth represents a potentially significant hazard.

10.2 The case for disinfection procedures at environmental sites and surfaces

The results of experiments on the survival and transfer of contamination involving worksurfaces, cloths, toilets and wastetraps together with investigations into the effectiveness of disinfectants as a means of reducing or preventing contamination transfer described in this thesis suggest that there are 3 basic points as summarized in Table 58 which need to be considered before sanctioning the use of disinfectants at environmental sites and surfaces.

Firstly, as already discussed, it is necessary to know the probability of significant contamination at the site or surface under consideration. In this context, it is important to consider both the likely levels of contamination (high, low or occasional) as well as the type of contamination, for example,

Table 58 An example of a Risk Evaluation for Environmental sites and surfaces as evaluated for the domestic environment (Scott et al 1982)

Site(s)	Frequency of occurrence of "significant" contamination	Risk of transfer of infection	Assessment of need for disinfection	Approach to disinfection
Reservoirs (Toilets, wastetraps etc.)	HIGH	RELATIVELY LOW - OCCASIONAL	RELATIVELY LITTLE except where known risk	CONTINUOUS RELEASE OR SUSTAINED ACTION DISINFECTANTS WHERE REQUIRED
Reservoir/disseminators (wet cloths & cleaning utensils)	HIGH	CONSTANT (i.e. all the time)	ALWAYS	USE DISPOSABLE OR DISINFECT IMMEDIATELY BEFORE USE
Contact Surfaces (eg food preparation and hand contact)	SOMETIMES	CONSTANT	ALWAYS	DISINFECT CRITICAL SURFACES IMMEDIATELY BEFORE AND BETWEEN USES

a predominance of potentially pathogenic Gram negative entero-bacteria or of harmless micrococci. The probability of significant contamination at reservoirs, reservoir/ disseminators and contact sites as determined by Scott et al (1982) is illustrated in Table 58.

Secondly, as discussed previously, it is also important to consider the likelihood that contamination may be transferred, either directly or indirectly, from one site to a higher risk site. The experiments described in this study indicate, for example, that under normal circumstances the risk of transfer from cloths and worksurfaces is very significant compared with toilets and wastetraps where it is relatively low or occasional. In the case of worksurfaces and other such contact surfaces, it is necessary to determine whether a surface is used in such a way as to make it a crucial contact site or is positioned in a high risk area either in food production, in manufacturing, or in hospital.

Thirdly, in conjunction with the first two considerations, the possibility of increased risks must be taken into account. For example, an outbreak of enteric infection involving spread of the disease by the faecal oral route would have particular implications for the disinfection of toilets and surrounding contact sites.

Having already taken these three points into consideration, it is then important to ensure that the disinfectant to be used is active and that the disinfection procedure is effective at the site or surface under consideration. Investigations described in this study suggest that the effectiveness of a disinfectant

in preventing transfer of contamination depends on two factors, namely, the activity of the disinfectant and the way in which it is applied and these factors must be carefully considered in the use of disinfectants. It was suggested earlier (Chapter 6 Page 136) that to be effective in preventing the transfer of contamination, a disinfectionprocedure must be capable of reducing contamination to a level of less than 10 organisms per contact area. Although some of the disinfection procedures studied here - drying, detergent wash followed by heat, wiping and chemical disinfectants - were capable of producing such satisfactory disinfection under certain conditions, the consistency with which disinfection was achieved was markedly affected by a number of different factors such as duration of action, presence of soil, the type of surface and bioburden etc. As a result, although recommended procedures might be appropriate under one set of conditions, they may prove ineffective in different circumstances. It is suggested that much further work is required to investigate the activity of disinfection procedures under "in use" or simulated "in use" conditions to define acceptable criteria for chemical disinfectants and other disinfection procedures.

The results of this study confirm that the removal of soil and reduction of the initial bioburden by "soap and water cleaning" before the application of a disinfectant has a significant effect on the reliability of the disinfection process, that is, the consistency with which satisfactory disinfection

is achieved. Although soap and hot water washing, together with rinsing may be regarded as an acceptable method of "disinfection" in terms of the definition (Chapter 1, Page 53) and is the method advocated in all situations for all items amenable to such treatment (ie in a sink, washing machine, dishwasher etc), the value of soap and water wiping for the disinfection of worksurfaces etc. must be questioned. The opinion of various workers is divided over whether soap and water is an effective method of disinfection; Werner (1975) and Ayliffe et al (1966) found that detergent or soap and water was generally less effective compared with other disinfectants, whereas Duppre (1975) showed that soap and water were equally effective as disinfectants in reducing bacterial contamination on floors. Thus, although it is accepted that soap and water cleaning will physically remove contaminated material (food particles, grease etc.), the assumption that decontamination of surfaces can be achieved by detergent cleaning is not upheld by the results in this study (Chapter 9). Furthermore, results suggest that detergent washing and rinsing is not a reliable method for the disinfection of cloths (Chapter 5 and Chapter 9). Further work is required to determine how and to what extent hot water and other types of detergents might be used to achieve effective decontamination of environmental sites and surfaces.

As already stated, the effectiveness of a disinfection procedure depends not only on the procedure itself but also on the way in which it is applied. One of the main aspects of the use of disinfectants which

is seldom fully appreciated is their limited duration of action. A number of examples of this have been reported in the literature including Ayliffe et al (1967) concerning cleaning of ward floors and Ojajarvi and Makela (1974) with regards disinfection of hospital surfaces. Previous studies of the home environment (Scott et al 1984) in which housewives were asked to apply disinfectants to 10 selected sites in the kitchen, bathroom and toilet clearly show that although hypochlorite and phenolic disinfectants produced rapid and effective decontamination of surfaces within 15 minutes of application, after a further 90 to 180 minutes, most sites had become re-contaminated. It is suggested that this re-contamination of environmental sites may occur in one or two ways: either as a result of re-use, or it may be due to re-growth of residual survivors not destroyed by the disinfection process. Laboratory experiments described in this thesis confirm the ability of organisms to proliferate at sites such as the toilet, sink wastetrap, cloths and to a limited extent also on dry surfaces. Disinfection studies with used contaminated cloths (Chapter 5 Page 125) showed that where cloths were not satisfactorily disinfected with sodium hypochlorite or Stericol and were subsequently allowed to remain damp, rapid re-growth of residual survivors occurred to give high levels of contamination of the order of 10^4-$10^5/cm^2$. Considering the various 'in-use' studies described in this thesis, the study of disinfectant impregnated

cloths (Chapter 9) indicates that where cloths are maintained in a satisfactory condition (in this case by the incorporation of disinfectant into the cloth) by ensuring the rapid and effective disinfection of the cloth after each use, then a substantial overall improvement in surface hygiene can be achieved.

By contrast, investigations of hospital toilets described in Chapter 8 confirm that daily disinfection with sodium hypochlorite or a quaternary ammonium product produces only limited reductions in microbial contamination. It is suggested that in this situation, re-contamination is associated with re-use of the toilet.

Such investigations of toilets and sink wastetraps suggest that for situations where disinfection is required to prevent transfer of contamination, this can only be achieved by use of a sustained action or continuous release disinfectant. Experiments described in Chapter 8 indicate that the installation of continuous release disinfectant units in toilets and wastetraps produced effective and sustained reduction in microbial contamination at these sites.

Finally, to reiterate, in the absence of a continuous release or sustained activity type agent, then it is crucial that the disinfectant is applied at the right time so that maximum disinfection cover can be achieved when required. It is suggested that to be effective in preventing transfer of contamination, disinfectants must be applied to contact surfaces immediately before each use.

Overall, it would appear that a successful and cost-effective disinfection policy as outlined by Daschner (1989) requires continuous assessment of environmental and other factors together with a flexible response. It is not sufficient to enforce either a simple 'always disinfect' or 'disinfectants are not required' approach to environmental disinfection, as neither of these attitudes serves the best interests of hygiene and infection control. Assuming that reliable and effective disinfectant products are available (and which are not environmetally toxic, Daschner 1989) and that the user understands the constraints of chemical disinfectants and the correct means of applying disinfectants, for example, the importance of soil removal, correct dilution etc., then there are some situations where a policy of effective disinfection should be continuously and rigorously enforced, for example, in food processing, special care units, operating theatres and the production of medicaments. There are also other situations where a flexible policy of disinfection should be adopted, for example, at toilets and floors etc.

In the home, adoption of effective and rigorous disinfection at food preparation sites could contribute to a reduction in food poisoning figures but disinfection of other environmental surfaces, including toilets, should be considered under an approach of flexible response.

For the specific sites considered in this thesis and with regard to food production, pharmaceutical production and hospitals, the following approach to disinfection is suggested.

Wet Cloths (and other wet cleaning utensils) in all situations must be maintained in a hygienic condition because they pose a constant risk as discussed earlier. The experiments on cloth disinfection indicate that the most reliable method is to wash soiled cloths and then dry them for a minimum of 2 hours at a high temperature, that is, 80°C or more. Chemical disinfectants, even at the concentrations recommended for soiled conditions cannot be relied upon to achieve adequate disinfection, particularly if cloths are to be stored before re-use. For a quick disinfection procedure applied immediately before cloths are to be used, sodium hypochlorite at a minimum of 4% v/v is recommended. Alternatively, single-use disposable cloths should be advocated for all situations where the risks of significant contamination and contamination transfer are high. Although studies have indicated the effectiveness of self-disinfecting cloth systems, studies in our laboratory (not included here) and by Babb et al (1981) have found such cloths to be unreliable when subject to misuse, as discussed earlier.

Toilets. Whilst the probability that toilets are contaminated may be high, the risk of transfer of contamination is low and therefore disinfection of toilets should be considered under a policy of flexible response. The application of toilet disinfection is probably of little value on a regular basis except in high risk situations, such as an outbreak of enteric infection. Studies into toilet disinfection indicate that where toilets are to be disinfected, the application

of chemical disinfectants, even on a daily basis, is likely to be of little vaue and that a continuous release type disinfectant is required in order to maintain a meaningful reduction. However, it is important that the use of a continuous release system should not lead to a complacent attitude towards hygiene. It must also be borne in mind that disinfection of the toilet itself produces only a limited reduction in contamination of surrounding sites and where decontamination of toilet seats, flush handles etc., is deemed necessary to prevent the transfer of contamination from these surfaces, then a direct application of disinfectant is required. Further studies are required to evaluate new developments in sustained-action disinfectants for toilets.

Wastetraps. Disinfection of wastetraps, like toilets, should be considered under a policy of flexible response and is probably only necessary in high risk areas such as operating theatres and special care units and pharmaceutical manufacture or when wastetraps are shown to be colonized with a potentially or known pathogenic species. As with toilets, a single application of chemical disinfectants provides only brief cover and a continuous release chemical disinfectant system or a procedure such as sterilizing electric elements is required if elimination of contamination is to be achieved.

Worksurfaces (and other hand and food contact surfaces). Although the frequency of contamination of such surfaces is relatively less than at reservoir and reservoir/ disseminator sites, experiments indicate that even

apparently dry and clean surfaces can harbour contamination in numbers sufficient to pose a risk of transfer of contamination via the hands or via inanimate objects following only brief contact. Therefore, contact surfaces must always be considered as a potential source of infection and again it is necessary to ensure that suitable measures are taken. As stated previously, the problems of re-contamination indicate that to be fully effective in preventing transfer of infection, contact surfaces require disinfection prior to each use. However, although disinfection of critical contact surfaces in areas such as food preparation and operating theatres before and between each use may be envisaged, for more general areas in kitchens, factories, wards, bathrooms and toilets etc., disinfection of contact surfaces before each use would not seem feasible. This being the case, the alternative approach which must be adopted is to recognise that effective sustained decontamination procedures are not generally available at the present time and to concentrate attention on recognizing the hazards and blocking routes of transfer via hands and inanimate objects. Such an approach requires thorough training of staff. Whereas the fact that toilets are a source of hand contamination is well established, the problem of persuading personnel that apparently clean, dry surfaces may sometimes be contaminated with sufficient organisms to allow transfer via the hands or an inanimate object is more difficult. Although the current attitude to discourage occasional or daily use of disinfectants

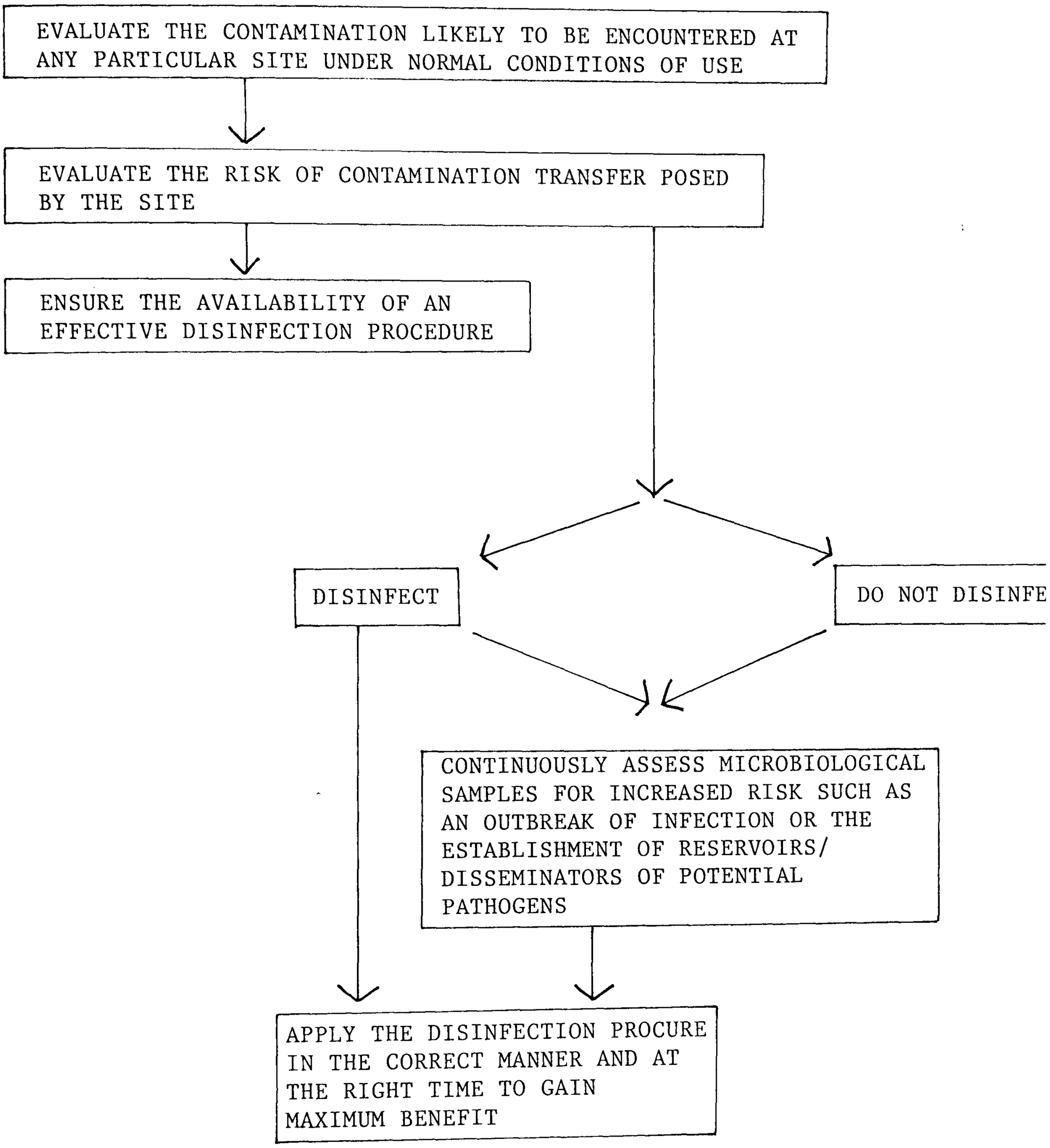
for general environmental sites and surfaces is valid on the grounds that it does little to prevent transfer of contamination except at the time of application, it is perhaps unfortunate that this policy has tended to encourage the assumption that such surfaces do not represent any infection hazard. Where infection outbreaks associated with transfer via the hands or inanimate objects are reported in the literature, there is little to indicate the original source of the contamination. It must be borne in mind that on occasion, infection may originate from a contaminated environmental site.

In conclusion, it would appear that for effective (and cost effective) infection control, those environmental (and other) sites and surfaces where a policy of rigorous disinfection is required must be delineated and suitably validated disinfection procedures (such as heat, chemical disinfectants, soap and water etc.) rigorously enforced.

For other sites and surfaces where the cost-benefits of routine disinfection are relatively low, (ie where cost-benefit considerations indicate that routine disinfection procedures are inappropriate) it is important that such sites are <u>not</u> dismissed as insignificant and a policy of "never disinfect" adopted. Personnel should be encouraged to adopt a policy of flexible response based on a clearly defined set of considerations as illustrated in the flow chart (Fig. 6). For all types of sites and surfaces in the general environment, the type of contamination

likely to be present and the risk of transfer of that contamination to a more crucial site is evaluated for so-called "normal conditions" and an effective disinfection procedure is determined for each and all of these sites and surfaces. Using such a risk evaluation, the decision whether to disinfect regularly, intermittently or not at all will be made. Where the risk posed is considered to be low and sites and surfaces are not disinfected or even when they are disinfected, but only intermittently, any increase in the risk factor, such as an outbreak of infection or the establishment of reservoirs and/or disseminators of potential pathogens, could result in the instigation of an appropriate and effective disinfection procedure.

Fig. 6 Flow chart of considerations for determining the use of disinfection procedures at environmental sites and surfaces



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APPENDIX 1

Neutralization trial for disinfected surfaces

The following trial was carried out to ensure that the incorporation of neutralizers into agar in contact plates (as recommended by Werner 1978 for surface disinfectant tests) did not inhibit bacterial growth. Use-dilutions of Stericol, Clearsol, Sodium hypochlorite and NADCC were prepared as described in Chapter 2.22. Eight separate 100ml drops of each diluted test agent and also sterile distilled water were pipetted onto laminate squares and stored in a lamina flow cupboard for 5 mins. After this period, the drops were contacted with agar contact plates (as described in 2.19) 4 of the contact plates contained nutrient agar alone and 4 contained nutrient agar plus 3% Tween 80, 0.3% lecithin and 0.5% sodium thiosulphate (Werner 1978). Suspensions of Staph. aureus (4163) and Ps. aeruginosa (6750) were prepared in sterile distilled water from overnight broth cultures (as described in 2.10 and 2.11) and further serially diluted in water . Following contact with the laminate squares, 4 separate 20μl drops of a 10^7 dilution of the bacterial suspensions were pipetted onto each contact plate and allowed to dry before incubation for 24h at 37°C. The trial was repeated 5 times for each test organism.

Results, showing the average count for 4 drops from a total of 20 contact plates containing either nutrient agar alone or nutrient agar plus neutralizers are given below.

The higher counts consistently achieved for agar plus neutralizers indicate the value of the incorporation of neutralizers for surface testing involving the

Neutralization trial for disinfected surfaces

a. Staph. aureus

Test agent	Average count from 4 x 20µl drops from a total of 20 contact plates	
	Nutrient agar	Nutrient agar and neutralizers
Water	67	77
Sodium hypochlorite (2,500 ppm)	53	69
NaDCC (2,500 ppm)	55	72
Stericol (2%)	56	65
Clearsol (1%)	61	71

b. Ps. aeruginosa

Test agent	Average count from 4 x 20µl drops from a total of 20 contact plates	
	Nutrient agar	Nutrient agar and neutralizers
Water	114	119
Sodium hypochlorite (2,500 ppm)	96	109
NaDCC (2,500 ppm)	80	107
Stericol (2%)	74	76
Clearsol (1%)	81	115

APPENDIX 2

STUDENTS' "t" TEST

Function: To assess whether the difference between the mean values of some parameter of sets of samples is statistically significant and not simply the result of chance. The null hypothesis (Ho) under test is that the two means are equal.

The observed deviation between the sample means is expressed in standard deviation units and it is then considered whether this value is greater or less than that which is conventionally accepted as reasonable for a t-variable.

Method:

$$t = \frac{\bar{x}a - \bar{x}b}{\sqrt{\frac{Sa}{Na} + \frac{Sb}{Nb}}} \quad (\text{where } \bar{x}a > \bar{x}b)$$

degrees of freedom = (Na-1) + (Nb-1)

where $\bar{x}$ = mean

S = standard deviation

N = sample size

t = student t, from tables of critical value of t

APPENDIX 3

Neutralization trial for disinfected liquids

The following trial was carried out to check for the carry over of chlorine from liquids treated with up to 10ppm available chlorine when attempting to recover bacterial contaminants.

A 10ppm available chlorine solution of sodium hypochlorine was prepared from a stock solution of sodium hypochlorite (B.D.H. Ltd., Dagenham) which contained 8.474% available chlorine (W/V).

Three sets of 6 test tubes were then set up as follows:

Set 1. each to contain 9ml of distilled water

Set 2. each to contain 9ml of sodium hypochlorite solution at 10ppm available chlorine

Set 3. each to contain 8.8ml of sodium hypochlorite solution (10ppm) plus 0.2ml of 0.1M sodium thiosulphate solution (May & Baker, Dagenham)

Using 1ml of an overnight broth culture of E. coli wt (as described in Chapter 2.2 and 2.10), a serial dilution was then performed on each set of tubes and total bacterial counts were confirmed using the Miles and Misra Method (Chapter 2.9).

Results showing the average count per 20μl drop and the total counts per ml for each set of tubes are given below

Test tube set	Dilution factor and cont per 20 μl drop		Total count per ml
	10^{-5}	10^{-6}	
Set 1.			
Untreated control Distilled water	TNTC	28	1.4×10^{9}
Set 2.			
10ppm available chlorine	0	0	0
Set 3.			
10ppm available chlorine + 0.2ml of 0.1M sodium thiosulphate	TNTC	25	1.25×10^{9}

The results indicated that for liquids treated with up to 10ppm available chlorine, the addition of 0.1M sodium thiosulphate was essential before attempting the recovery of bacteria and that when added in volumes of 0.2ml to a total of 10ml, the addition of 0.1M sodium thiosulphate caused little inhibition.

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The survival and transfer of microbial contamination via cloths, hands and utensils

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Survival and transfer of bacteria from laminated surfaces and cleaning cloths were investigated under laboratory conditions. Drying produced substantial reductions in numbers of recoverable organisms and achieved satisfactory decontamination of clean laminate surfaces. On soiled surfaces and on clean and soiled cloths, Gram-positive and some Gram-negative species survived for up to 4 h, and in some cases up to 24 h. Where contaminated surfaces or cloths came into contact with the fingers, a stainless steel bowl, or a clean laminate surface, organisms were transferred in sufficient numbers to represent a potential hazard if in contact with food.

Previous studies of the domestic kitchen indicate that wet cloths and cleaning utensils, together with hand and food contact surfaces, are important elements in cross-contamination (Scott *et al.* 1981).

The occurrence of contaminated wet cloths has been investigated. Scott *et al.* (1982) found that 48% and 52% respectively of domestic dishcloths and cleaning cloths showed counts greater than 100 organisms/20 cm^2, 30% and 26% indicating the presence of enterobacteria. De Wit *et al.* (1979) showed that, during preparation of chickens contaminated with *Escherichia coli*, K12 indicator organisms, 74% of kitchen dishcloths, together with other kitchen surfaces, became contaminated. Tebutt (1986) found that 74% of cloths used for wiping food shop surfaces were contaminated with one or more of the following organisms: *E. coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *Clostridium perfringens*. *Escherichia coli* was isolated from 56% of cloths, nearly half containing more than 10^5 colonies. Davis *et al.* (1968), Gilbert (1969), Mackintosh & Hoffman (1984) and Tebutt (1986) showed that wiping hard surfaces with contaminated cloths may result in contamination of hands, equipment and other surfaces.

Surface contamination in kitchens has also been investigated. Scott *et al.* (1982) found that 10 to 24% of domestic kitchen worktops, chopping boards, refrigerator and cooking hob surfaces were contaminated with >200 organisms/20 cm^2, 10–20% showing contamination with enterobacteria. Studies in Dutch homes, where infant salmonellosis was identified, showed that kitchen sinks and work surfaces were frequently contaminated with the infecting serotype (van Schothurst *et al.* 1978). Mendes *et al.* (1978) showed that 56% of bacteria isolated from surfaces in catering establishments were of faecal origin, including *Salmonella* spp. isolated from food preparation and hand contact sites.

Despite these investigations, little attempt has been made to quantify transfer risks from contaminated surfaces. In this paper we describe laboratory experiments which determine the extent to which survival of organisms on cloths and laminate surfaces may be associated with cross-contamination via the hands, cloths and stainless steel surfaces.

Materials and Methods

MEDIA AND SOLUTIONS

Culture media and solutions were prepared from media bases (Oxoid).

TEST ORGANISMS

Laboratory strains (ls) were *Escherichia coli* NCTC 8196; *Klebsiella aerogenes* NCTC 5055; *Pseudomonas aeruginosa* NCTC 6749; *Salmonella abony* NCTC 6017; and *Staphylococcus aureus* NCTC 4163. Wild type (wt) strains were of environmental origin including *E. coli* (API 20E profile 1044512), *Salmonella* spp. (API profile 6504512) and *Staph. aureus* (coagulase and DNase positive).

PREPARATION OF TEST SUSPENSIONS

Tryptone soya broth suspensions as described by Werner (1975) were used to simulate soiled conditions. Broth cultures were grown in this medium at 37°C for 18 h and diluted with broth to give the required number of organisms/ml. To simulate clean conditions, broth cultures were centrifuged, washed twice and diluted in distilled water to give the required number of organisms/ml. Suspensions were standardized by the method of Miles *et al.* (1938).

LAMINATE SURFACES AND CLEANING CLOTHS

White laminate surfaces (20 cm^2), chosen to represent commonly encountered work surfaces, were bonded with contact adhesive on to stainless steel to prevent bowing. Surfaces were swabbed with 70% ethanol before use. Cloths were of the dry-woven 'J-cloth' type (Paynes Scientific, Slough). Clean cloths were soaked in 0·9% saline for 10 min. Elworthy & Graham (1969) showed that pre-wetting, allowing the saline to fill the capillary network of the cloths, can substantially reduce adsorption of contamination. Soiled cloths were cloths used for washing-up in domestic kitchens for 3 days. Clean and soiled cloths were cut into portions (1260 cm^2), and sterilized in glass Petri dishes (121°C for 15 min).

SURVIVAL ON CLEAN AND SOILED LAMINATE SURFACES AND CLOTHS

Bacterial test suspensions (100 μl) in water or broth were pipetted on laminate surfaces to give a total inoculum size of about 300 organisms. Plates were stored in assay dishes at 30°C and 40–45% RH and the drops dried in 90 min. Clean and soiled cloths were inoculated with 3 ml of test suspensions in water to give a total inoculum of about 120 organisms/cm^2. Cloths were stored in closed Petri dishes at room temperature (18–20°C) and 60% RH. After 48 h the cloths were dry to the touch. Cloths and surfaces were sampled and numbers of survivors determined as described below.

TRANSFER FROM SOILED LAMINATE SURFACES VIA THE FINGERTIPS AND A STAINLESS STEEL BOWL

Laminate surfaces were inoculated with broth suspensions of test organisms as described above. Transfer from surfaces via fingertips (middle and forefinger of the right hand) or a stainless steel bowl (representing a piece of kitchen equipment) was determined at time 0 (immediately after inoculation) and at 1, 2 and 24 h. The fingertips and the bowl were swabbed with 70% ethanol which was allowed to evaporate before contact with laminate surfaces. The fingertip or bowl was placed in contact with the whole of an inoculated drop for 30 s, during which time the bowl was weighted with a 200 g weight. Surfaces of the fingertips and the stainless steel bowl were sampled and the numbers of organisms transferred determined as described below.

TRANSFER FROM CLOTHS TO FINGERS AND LAMINATE SURFACES

Soiled cloths were inoculated with broth suspensions of test organisms as described above. Transfer of organisms to the fingertips and to a laminate surface was determined at time 0 and 1, 4 and 24 h after inoculation. The fingertips (middle and forefinger of the right hand) were swabbed with 70% ethanol which was allowed to evaporate before contact with cloths. The fingertips were placed firmly in contact with cloths for 30 s. Cloths were then used to wipe a sterile laminate surface. The fingertips and the laminate surface were then sampled and the

Table 1. Survival of organisms on clean and soiled laminate surfaces

	Number of colony-forming units recovered per 25 cm² contact plate																	
	Escherichia coli †		*Escherichia coli* *		*Klebsiella aerogenes*†		*Klebsiella pneumoniae**		*Pseudomonas aeruginosa*†		*Salmonella abony*†		*Salmonella* spp.*		*Staphylococcus aureus* †		*Staphylococcus aureus* *	
Soiled conditions																		
Total surface inoculum cfu/100 μl	250	200	250	250	300	120	370	—	200	170	400	350	300	—	320	550	204	500
Recovery times (h)																		
0	153	49	193	85	52	37	225	—	164	67	167	17	190	—	125	149	116	125
1	200	7	248	79	85	40	268	—	156	36	162	14	199	—	147	118	157	146
4	0	4	20	2	4	3	35	—	2	12	20	3	37	—	111	80	37	160
24	<1	2	3	0	0	0	0	—	1	5	0	3	13	—	42	27	78	7
Clean conditions																		
Total surface inoculum cfu/100 μl	300	260	290	100	437	110	150	—	150	140	300	400	375	—	190	100	200	500
Recovery times (h)																		
0	240	84	181	49	52	41	44	—	108	44	112	18	125	—	83	24	141	176
1	157	38	172	47	61	22	4	—	94	24	103	17	131	—	75	12	193	102
4	0	0	0	3	0	1	1	—	3	0	0	0	1	—	2	3	7	2
24	0	0	1	0	0	0	3	—	1	0	0	0	1	—	0	1	5	0

† Laboratory strains; * wild-type strains.

Table 2. Survival of organisms on clean and soiled cloths

	Number of colony-forming units recovered per 25 cm² contact plate																	
	Escherichia coli †		*Escherichia coli* *		*Klebsiella aerogenes*		*Klebsiella pneumoniae**		*Pseudomonas aeruginosa*†		*Salmonella abony*†		*Salmonella* spp.*		*Staphylococcus aureus* †		*Staphylococcus aureus* *	
Soiled conditions																		
Inoculum per 25 cm² of cloth	2225		7075		2675		8750		3125		4350		7800		36 662		5823	
Recovery times (h)																		
0	217	T	T	T	T	T	T	180	T	T	180	T	T	T	T	T	T	T
1	77	41	T	T	T	T	165	45	T	T	50	T	100	135	T	T	154	60
4	50	37	175	210	161	241	73	9	T	T	33	100	52	71	T	91	2	23
24	0	0	T	68	22	T	T	T	T	T	7	15	4	7	35	6	5	8
48	0	0	T	45	T	T	T	T	T	T	3	3	1	0	5	0	0	0
Clean conditions																		
Inoculum per 25 cm² of cloth	2375		5750		2825		1750		4912		3937		7600		22 500		4325	
Recovery times (h)																		
0	146	174	T	T	100	124	250	245	226	161	143	170	T	T	T	T	T	T
1	121	105	T	T	28	51	12	20	77	47	54	46	T	T	T	T	46	92
4	8	21	231	225	36	29	1	1	39	23	30	43	204	134	T	472	12	11
24	0	2	20	3	1	9	0	0	T	T	14	23	27	12	135	45	1	1
48	0	0	18	3	T	T	0	0	T	T	5	8	15	2	9	7	0	0

† Laboratory strains; * wild type strains; T, too numerous to count.

Table 3. Transfer of organisms from a soiled laminate surface to the fingers or a stainless steel bowl

	Number of colony-forming units recovered per 25 cm^2 contact plate											
	Fingertip						Stainless steel bowl					
	*Escherichia coli**		*Salmonella* spp.*		*Staphylococcus aureus**		*Escherichia coli**		*Salmonella* spp.*		*Staphylococcus aureus**	
Total surface inoculum cfu/100 μl	330	200	270	560	210	210	330	200	270	560	210	210
Recovery times (h)												
0	59	55	42	59	57	49	59	53	49	73	38	46
1	69	62	55	78	99	85	56	49	54	95	46	40
2	25	80	<1	11	46	82	30	17	31	0	1	34
24	1	5	0	6	18	20	0	0	0	0	0	3

* Wild type strains.

numbers of organisms transferred determined as described below.

CONTACT PLATE SAMPLING

Surfaces were sampled with tryptone soya agar contact plates which were air-dried in a laminar flow cabinet for 1 h. For sampling of laminate and cloth surfaces, plates were weighted with 200 g weights and left in contact for 30 s. Fingertips were pressed on the surface of contact plates for 30 s. Although there is evidence that recovery may be less than by other methods (Angelotti *et al.* 1964; Gilbert 1970; Scott *et al.* 1984) contact plate sampling was considered the most appropriate method for this investigation which is concerned with contamination transfer from contact between surfaces.

Tryptone soya plates were incubated (37°C 24 h) and the number of colony-forming units (cfu) per plate (25 cm^2) recorded. More than 250 cfu was recorded as 'too numerous to count'. Results were assessed from the average of two plate counts at each sampling time. Experiments were carried out twice. Although there was reasonable agreement between duplicate counts for a particular experiment, in some cases considerable differences were observed between experiments. This lack of reproducibility is a common feature associated with surface sampling (Pettit & Lowbury 1968; Werner *et al.* 1977). Results were therefore used to identify situations where significant contamination *may* be encountered.

Results

SURVIVAL ON LAMINATE SURFACES

Results given in Table 1 indicate that a substantial proportion of the inoculum was recovered at 0 and 1 h from both clean and soiled surfaces, during which time surfaces remained damp, although for some organisms, particularly on clean surfaces, lethal effects were immediately apparent. Some species showed an initial increase in numbers between 0 and 1 h suggesting multiplication on surfaces. For clean surfaces there was little survival at 4 and 24 h, but under soiled conditions *E. coli* (wt), *Kl. pneumoniae* (wt), *Salm. abony* (ls) and *Salmonella* spp. (wt) survived in significant numbers up to 4 h, and up to 24 h for *Staph. aureus* (ls and wt).

SURVIVAL ON CLOTHS

Results given in Table 2 indicate that although numbers of organisms on clean cloths declined over the drying period, with the exception of *Kl. pneumoniae* (wt) and *Staph. aureus* (wt), recovery at 4h was greater than 20 cfu/25 cm^2. At 24 h, the majority of clean cloths contained less than 20 cfu/25 cm^2 , but for *Kl. aerogenes* (ls) and *Ps. aeruginosa* (ls), there was regrowth of residual survivors. Soiled cloths showed somewhat higher contamination with only *Staph. aureus* (ls and wt), *Salmonella* spp. (ls and wt) and *E. coli* (ls) reduced to less than 20 cfu per 25 cm^2. For the remaining species, although there was an initial reduction at 4 h, substantial regrowth of residual survivors occurred within 24 h.

TRANSFER FROM A SOILED LAMINATE SURFACE

Contamination transferred from a soiled laminate surface via the fingertips (total surface contact area approx. 2 cm^2) or a stainless steel bowl (surface contact area approx. 1 cm^2) is shown in Table 3. Test organisms were those

which showed significant survival on laminate surfaces for up to 4 h. Significant numbers of all three organisms were transferred for 1-2 h after contamination and up to 24 h for *Staph. aureus* via the fingertips.

TRANSFER FROM CLOTHS

Contamination transferred to fingertips or a laminate surface from contaminated soiled cloths is shown in Table 4. Apart from *E. coli*, transferred to fingertips, and *Staph. aureus*, transferred to laminate surface, significant numbers of all three organisms were transferred for up to 48 h after inoculation. Where numbers of organisms increased over 4–48 h due to regrowth of residual survivors (as indicated in Table 2) this was accompanied by increased transfer.

Discussion

Results from the first part of this investigation illustrate the extent of bacterial survival and growth on laminate surfaces and cleaning cloths. Drying, as expected, produced substantial reductions in recoverable organisms and, for clean laminate surfaces, achieved satisfactory decontamination, but Gram-positive and some Gram-negative species were recoverable in significant numbers from soiled surfaces and from clean and soiled cloths for up to 4 h and in some cases up to 24 and 48 h. For cleaning cloths, certain organisms showed initial decline in numbers followed by a subsequent increase indicating adaptation of organisms enabling multiplication on relatively dry cloths.

The results suggest that bacterial survival and regrowth on surfaces depends on a number of factors and is largely unpredictable. Recovery from cloths was generally higher than from surfaces (except for *Staph. aureus*). This probably relates to the higher inoculum size used and the slower drying rate of the cloths. As reported by Lowbury & Fox (1953) and Rathmachers & Borneff (1977) soiling is an important factor in preserving viability on hard surfaces. For cleaning cloths, soiling also encouraged regrowth of residual survivors although for *Kl. pneumoniae* (ls) and *Ps. aeruginosa* (ls) regrowth occurred with clean as well as soiled cloths. Although, as found by Pettit & Lowbury (1968) and Rathmachers & Borneff (1977), survival of Gram-positive species on laminate surfaces was greater than that of Gram-negative organisms, this was not the case with cleaning cloths. This may be due to differences in drying rate and nutrient availability between hard and cloth surfaces; Rathmachers & Borneff (1977) suggest that survival of *Staph. aureus* in moist situations under conditions of nutrient limitation may be less than that of Gram-negative species. When Gram-negative species were compared, it was found that *E. coli* (ls) was particularly sensitive to drying on both cloths and laminate surfaces, but survival of other Gram-negative organisms varied according to the nature of the surface and the presence or absence of soil. Some differences between laboratory and wild strains were observed, but there was no pattern which might suggest that wild strains should be used in preference to laboratory strains for assessing surface hygiene in laboratory experiments.

Table 4. Transfer of organisms from a soiled cloth to the fingers or a work surface

	Number of colony-forming units recovered per 25 cm² contact plate											
	Fingertip						Laminate surface					
	*Escherichia coli**		*Klebsiella aerogenes*†		*Staphylococcus aureus**		*Escherichia coli**		*Klebsiella aerogenes*†		*Staphylococcus aureus**	
Total inoculum per 25 cm² cloth	976		714		2976		976		714		2976	
Recovery times (h)												
0	5	8	48	92	101	113	31	37	41	43	35	31
1	5	7	60	34	77	153	22	31	34	39	28	27
4	4	3	41	24	91	57	20	22	22	24	8	8
24	T	T	T	T	T	T	T	T	T	T	T	T
48	T	T	T	T	T	T	T	T	T	T	T	T

† Laboratory strains; * wild type strains; T, too numerous to count.

In the second part of this investigation contamination transfer by the fingers, cloths or a stainless steel surface was studied. Results suggest that, where contaminated surfaces come into even relatively brief contact with the fingers or an inanimate surface, significant numbers of organisms can be transferred which are recoverable onto an agar surface. Although transfer was reduced following storage of laminate surfaces, surface survival up to 2 h for all three species and up to 24 h for *Staph. aureus* was sufficient to allow significant transfer by the fingers or a stainless steel bowl. Similarly, where contaminated cloths were handled or applied to a clean laminate surface, significant transfer occurred. Transfer from cloths was generally greater than from laminate surfaces, giving contamination levels 'too numerous to count' at 24 and 48 h. This correlates with higher contamination levels occurring in stored cloths. For transfer experiments (Table 4) soiled cloths were inoculated with broth rather than aqueous suspensions of test organisms as used for survival studies (Table 2); results not reported here showed that the additional soiling facilitated the regrowth of *E. coli*, *Staph. aureus* and *Salmonella* spp. in addition to *Klebsiella* spp. and *Ps. aeruginosa*.

In assessing hazards of cross-contamination, it must be borne in mind that contamination applied to cloths and surfaces in these investigations was relatively low compared with levels which have been reported under in use conditions. Investigations suggest that in use contamination of cloths may range from 10^3 to 10^8 organisms/cm^2 (Davis *et al.* 1968, Mendes *et al.* 1978, Scott *et al.* 1982), whilst Gilbert & Maurer (1968) and Gilbert (1970) suggest that surface contamination following contact with food may be of the order 10^2–10^6 organisms/cm^2. Clinical investigations indicate that infection risks depend on numbers of organisms transferred. McCullough & Eisele (1951) reported that the infective dose of salmonellas may be 10^6 organisms or much lower. Outbreaks involving chocolate and Cheddar cheese suggests that the infective dose may be as little as 50–100, and less than 10 organisms respectively (Gill *et al.* 1983; Greenwood & Hooper 1983; D'Aoust 1985). For toxin-producing species such as *Bacillus aureus*, *Clostridium perfringens* and *Staph. aureus*, it is accepted that the infective dose is greater than 10^6/g food, but transfer of even small numbers of these organisms to food increases the risk of multiplication to hazardous levels under conditions of poor storage etc.

From laboratory experiments described here it is concluded that where contaminated surfaces or cloths containing even relatively low numbers of organisms come into contact with the fingers and other surfaces (e.g. a stainless steel bowl or a clean laminate surface) organisms may be transferred in sufficient numbers to represent a potential infection hazard. Although drying plays an important part in maintenance of hygiene in the kitchen and other environments, drying *per se* cannot be relied upon to prevent transfer of infection from laminate and cloth surfaces involved in potentially hazardous situations. The investigation emphasizes the importance of good hand hygiene and adequate decontamination procedures applied to cloths, laminate surfaces, utensils and other food contact surfaces during handling and/or preparation of food and in other critical environments. Methods for decontamination of cloths and laminate surfaces are currently under investigation.

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Investigations of the effectiveness of detergent washing, drying and chemical disinfection on contamination of cleaning cloths

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Detergent washing, drying and chemical disinfection for decontamination of cleaning cloths was investigated with cloths contaminated by use in the domestic environment. Detergent washing produced only limited reductions in microbial contamination and cloths then stored at room temperature for 24 h showed increases in contamination due to multiplication of residual survivors. For effective and consistent decontamination of cloths, detergent washing followed by drying at 80°C for 2 h was required. Hypochlorite and phenolic disinfectants produced significant reductions in contamination, but chemical disinfection may be unreliable where cloths are heavily contaminated.

Heavy contamination of wet mops and cleaning utensils and the potential for spread of such contamination has been recognized by several workers. In domestic dishcloths, Davis *et al.* (1968) reported total counts of up to 10^8 organisms/cloth with up to 10^7 *Escherichia coli*. Heavy contamination of wet cloths and cleaning utensils in hospitals with enterobacteria and *Pseudomonas aeruginosa* was reported by Westwood *et al.* (1971), Whitby & Rampling (1972) and Baird *et al.* (1976).

Although reusable wet cleaning cloths are now discouraged in UK hospitals, these items are still in use. Most particularly, cloths are used in domestic and other food preparation environments. A survey of domestic dishcloths and cleaning cloths by Scott *et al.* (1982) showed that about 50% had counts of greater than 100 cfu/25 cm^2. Organisms included *E. coli*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Pseudomonas* spp., *Staphylococcus aureus* and streptococci. Similar results were reported by Finch *et al.* (1978).

Only limited data have been published which can be used to formulate effective policies for decontamination and maintenance of re-usable cleaning utensils (Westwood *et al.* 1971; Walter & Schillinger 1975; Scott *et al.* 1984). Recent laboratory investigations (Scott & Bloomfield 1989) showed that although drying at room temperature produces substantial reductions in recoverable organisms from artificially contaminated cloths, Gram-negative and Gram-positive species may survive for up to 4 h and in some cases up to 24 h. Although there may be an initial decline in numbers for Gram-negative species this is followed by an increase on cloths which may be relatively dry.

Experiments confirmed that where contaminated cloths come into contact with the fingers or a clean surface organisms may be transferred in sufficient numbers to represent an infection hazard if in contact with food. Transfer by contact with contaminated cloths has also been reported by Babb *et al.* (1981) and Mackintosh & Hoffman (1984). These studies emphasize the

importance of decontamination procedures for reusable cloths in kitchens or environments where cross-contamination represents a hazard.

In this paper, washing, drying and disinfection procedures for decontamination of cloths contaminated by use in the domestic environment are investigated.

Materials and Methods

MEDIA AND SOLUTIONS

Media and solutions were prepared from media bases (Oxoid). Neutralization medium, prepared as described previously (Scott *et al.* 1984), contained Tween 80, 3% w/v; lecithin 0·3% w/v; l-histidine 0·1% w/v and sodium thiosulphate 0·5% w/v in quarter-strength Ringer solution. Tests confirmed that the medium produced satisfactory neutralization under appropriate conditions.

PREPARATION OF CONTAMINATED CLOTHS

Cloths were of the dry-woven 'J-cloth' type (Paynes Scientific, Slough). Volunteers used the cloths as 'washing up' cloths etc in the domestic kitchen for 3 d and then returned them to the laboratory for immediate investigation. Cloths were not to be used in combination with chemical disinfectants.

DETERGENT AND DISINFECTANT SOLUTIONS

Sodium hypochlorite solution containing 10–14% w/v available chlorine ($AvCl_2$) (BDH), Stericol (Sterling Industrial Ltd, Sheffield) and a proprietary anionic liquid detergent (Waitrose Ltd) were diluted as required in sterile distilled water. Hypochlorite disinfectant was standardized by thiosulphate titration.

RINSE METHOD FOR ESTIMATION OF TOTAL COUNTS IN CLOTH PORTIONS

Cloth portions were transferred to 100 ml of quarter-strength Ringer solution or neutralizing medium in 250 ml conical flasks. The flasks were shaken on a mechanical shaker for 10 min to release organisms from cloths. Serial dilutions of the rinse fluid were prepared in quarter-strength Ringer solution and colony forming units (cfu) determined on tryptone soya agar by the method of Miles *et al.* (1938). Results are expressed as the total count/cm^2 of cloth.

DETERGENT WASH AND DRYING OF CLOTHS

Cloths were divided into four portions (approx. 325 cm^2). One portion was immediately transferred to quarter-strength Ringer solution to determine the total count. The remaining portions were washed in anionic detergent (diluted to 1·2% v/v) and rinsed in tap water. One portion was transferred to quarter-strength Ringer solution for determination of total count, whilst the remaining two portions were stored in glass Petri dishes for 24 h before performing total counts. For one cloth, the Petri dish was maintained with the lid closed at room temperature, 30% r.h., whilst the other was maintained with the lid ajar at 50°C, 50% r.h. To examine the effect of drying at elevated temperature, cloths were divided into five portions (approx. 200 cm^2). One portion was used to determine the initial viable count whilst the remaining portions were washed in anionic detergent, rinsed in tap water and stored in glass Petri dishes (lid ajar) at 80°C, $<5\%$ r.h. for up to 24 h. At intervals cloth portions were transferred to quarter-strength Ringer solution for total counting.

CHEMICAL DISINFECTION OF CLOTHS

Cloths were aseptically divided into five portions (approx. 200 cm^2). One portion was transferred to neutralization medium to determine the initial count. Two of the remaining cloth portions were immersed in 2% v/v Stericol and two in sodium hypochlorite solution, 4000 ppm $AvCl_2$. After 2 min, the cloth portions were rinsed in running water for 30 s and one cloth from each disinfectant treatment was transferred to 100 ml of neutralization medium for determination of total counts. The remaining two cloth portions were wrung out, folded and stored in glass Petri dishes for 24 h and then transferred to neutralization medium for total counting. To examine the effect of neutralization of cloths in addition to rinsing after disinfectant treatment, the above procedure was repeated with the following modification. After disinfectant and rinsing treatments, cloth samples to be stored

Table 1. Total counts recovered from contaminated cloths after detergent wash and rinse and low temperature drying

	Total count per cm^2 cloth			
Cloth sample	Initial count	Detergent wash and rinse	Detergent wash and rinse, stored 24 h at room temperature	Detergent wash and rinse, stored 24 h at 50°C
1	$6{\cdot}5 \times 10^2$	$7{\cdot}0 \times 10^1$	$2{\cdot}3 \times 10^3$	3
2	$3{\cdot}3 \times 10^2$	$8{\cdot}4 \times 10^1$	$2{\cdot}2 \times 10^3$	3
3	$3{\cdot}0 \times 10^3$	$4{\cdot}7 \times 10^2$	—	2
4	$>3{\cdot}2 \times 10^3$	$6{\cdot}2 \times 10^2$	$2{\cdot}6 \times 10^3$	$7{\cdot}0 \times 10^1$
5	$1{\cdot}7 \times 10^2$	$7{\cdot}6 \times 10^2$	$8{\cdot}8 \times 10^2$	0
6	$1{\cdot}9 \times 10^3$	$1{\cdot}5 \times 10^3$	$2{\cdot}3 \times 10^3$	$2{\cdot}6 \times 10^1$
Mean count	$1{\cdot}6 \times 10^3$	$5{\cdot}8 \times 10^2$	$2{\cdot}0 \times 10^3$	$1{\cdot}7 \times 10^1$

for 24 h were immersed in neutralizing medium for 10 min. They were then wrung out and folded before transfer to Petri dishes for storage at room temperature and sampling as described above.

Results

DETERGENT WASH AND DRYING OF CLOTHS

Results given in Table 1 indicate that following detergent wash and rinse treatment, the mean total count was reduced from 1600 to 580 cfu/cm^2. This reduction was significant at the $P < 0{\cdot}2$ level although in one sample, the washing process produced some increase in contamination. Cloth portions stored at room temperature for 24 h showed some increase in average total count to 2000 cfu/cm^2 but this increase was not significant compared with counts determined prior to treatment. Drying cloths at 50°C for 24 h produced significant reductions in total counts but contaminants were recoverable from four of six cloths and the highest count was 70 cfu/cm^2. The results of detergent wash and drying at 80°C (Table 2) indicate that whereas drying at 80°C for 1 h produced total elimination of contamination from all but one cloth, drying at 80°C for 2 h or more was effective for all cloths.

Table 2. Total counts recovered from contaminated cloths after detergent wash and rinse and drying at 80°C

	Total count per cm^2 cloth				
		Detergent wash and rinse followed by drying at 80°C for			
Cloth sample	Initial count	1 h	2 h	3 h	4 h
1	$1{\cdot}0 \times 10^5$	0	0	0	0
2	$8{\cdot}8 \times 10^4$	<10	0	0	0
3	$3{\cdot}5 \times 10^4$	0	0	0	0
4	$5{\cdot}5 \times 10^4$	0	0	0	0
5	$2{\cdot}5 \times 10^4$	0	0	0	0

CHEMICAL DISINFECTION OF CLOTHS

Effects of chemical disinfection on contamination of cloths are shown in Table 3. There were no detectable survivors in five of 13 cloths after treatment with 2% (v/v) Stericol (2 min contact period). For the remaining eight cloths, although reductions were achieved, total counts in excess of $10^3/cm^2$ were recorded. The efficiency of the disinfection procedure was related to initial contamination levels; for the five cloths which were satisfactorily disinfected, initial counts were 10^2–$10^3/cm^2$ compared with 10^4–10^6 for cloths where satisfactory disinfection was not observed. For all nine cloths stored without neutralization of residual Stericol, the initial reduction was maintained over 24 h, indicating some further activity. For two of these cloths, a further reduction to give no detectable survivors was achieved. Where Stericol was neutralized before storage, regrowth of residual survivors occurred in all four cloths even though a zero count had been previously recorded in two cloths.

Sodium hypochlorite solution, 4000 ppm $AvCl_2$, achieved somewhat better results, with no detectable survivors in 10 of 13 cloths immediately after disinfection. For the remaining three cloths, although reductions occurred,

Table 3. Total counts recovered from contaminated cloths after disinfection with hypochlorite 4000 ppm available chlorine and Stericol 2% v/v

	Total count per cm^2 cloth				
	Stericol 2% v/v			Hypochlorite 4000 ppm $AvCl_2$	
Cloth sample	Initial count	After treatment	After treatment and 24 h storage	After treatment	After treatment and 24 h storage
1	$5{\cdot}0 \times 10^2$	0	0	0	0
2	$7{\cdot}5 \times 10^2$	0	0	0	0
3	$1{\cdot}0 \times 10^3$	0	0	0	0
4	$8{\cdot}5 \times 10^4$	$2{\cdot}5 \times 10^4$	$4{\cdot}5 \times 10^4$	0	0
5	$4{\cdot}0 \times 10^5$	$2{\cdot}5 \times 10^5$	$2{\cdot}5 \times 10^5$	$2{\cdot}5 \times 10^2$	$8{\cdot}5 \times 10^4$
6	$5{\cdot}0 \times 10^5$	$5{\cdot}0 \times 10^4$	0	0	0
7	$6{\cdot}0 \times 10^5$	$3{\cdot}0 \times 10^5$	$2{\cdot}5 \times 10^5$	0	$5{\cdot}0 \times 10^3$
8	$1{\cdot}1 \times 10^6$	$5{\cdot}0 \times 10^3$	$2{\cdot}5 \times 10^2$	$2{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^4$
9	$2{\cdot}5 \times 10^6$	$3{\cdot}0 \times 10^3$	0	0	0
	Initial count	After treatment	After treatment, neutralization and 24 h storage	After treatment	After treatment neutralization and 24 h storage
10	$5{\cdot}0 \times 10^2$	0	$1{\cdot}1 \times 10^5$	0	$1{\cdot}0 \times 10^3$
11	$2{\cdot}0 \times 10^3$	0	$3{\cdot}0 \times 10^5$	0	$3{\cdot}7 \times 10^3$
12	$3{\cdot}7 \times 10^4$	$2{\cdot}5 \times 10^3$	$8{\cdot}0 \times 10^4$	0	$4{\cdot}5 \times 10^3$
13	$6{\cdot}5 \times 10^4$	$3{\cdot}0 \times 10^4$	$3{\cdot}0 \times 10^5$	$2{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^5$

counts of 10^2–$10^3/cm^2$ were recorded. For the nine cloths stored without neutralization of residual hypochlorite, zero counts were recorded at 24 h for six cloths, but for three cloths regrowth of residual survivors occurred, indicating little or no further action. For the four cloths where hypochlorite was neutralized before storage, regrowth of residual survivors occurred even though a zero count had been previously recorded in three cloths.

Discussion

Although assessment of in use contamination was not the primary aim of this investigation, results confirm that cloths may become heavily contaminated during use in the domestic environment. Tables 1–3 show initial counts of $1{\cdot}7 \times 10^2$ to $2{\cdot}5 \times 10^6$ cfu/cm^2 from cloths returned to the laboratory after 3 d use.

In the first part of this investigation detergent washing, rinsing and drying of cloths were investigated. Detergent washing and rinsing produced limited reductions in microbial contamination and where cloths were stored at room temperature for 24 h, during which time they remained damp, an increase in contamination was usually observed indicating multiplication of organisms. When the drying temperature was raised to 50°C, this produced a significant reduction in cloth contamination at 24 h, but this method cannot be considered as a reliable decontamination method, since three of the seven cloths showed contamination. When the drying temperature was increased to 80°C, effective decontamination was consistently achieved within 2 h.

Results from the second part of this investigation identify problems on using chemical disinfectants for decontamination of cloths. For this investigation, a phenolic and hypochlorite disinfectant, the types most commonly used for general disinfection of surfaces in the domestic and other environments, were chosen. Although hypochlorite achieved better initial results than the phenolic, producing no detectable survivors in 10 of 13 cloths, compared with five of 13 cloths for the phenolic, neither disinfectant can be relied upon to produce consistently satisfactory decontamination, particularly with heavily contaminated cloths. The results indicate the potential hazard of using disinfectants for

decontamination of cloths prior to storage; for 12 of 26 cloth portions examined, storage produced regrowth of residual survivors even though some cloths were apparently 'sterile' after disinfectant treatment. This occurred particularly with hypochlorite-treated cloths where residual hypochlorite would be rapidly destroyed during drying. Regrowth was observed in all cloth portions where residual disinfectant was neutralized before storage.

From the results of this investigation, we conclude that, for effective treatment of cleaning cloths, heat must be regarded as the most reliable method, using a minimum temperature of 80°C for 2 h. Where chemical disinfection is required for rapid decontamination, this must be done only immediately before, or between, activities. It must be borne in mind that chemical disinfection may be unreliable for heavily contaminated cloths, which should be given a detergent wash and rinse to reduce the initial bioburden before disinfection. Use of disposable cloths should be encouraged wherever possible, particularly in association with food preparation and other environments such as hospitals where contamination transfer represents a particular risk.

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The survival of microbial contamination on worksurfaces and cloths and its transfer via cloths, hands and utensils

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Survival and transfer of bacteria from work surfaces and cleaning cloths were investigated in the laboratory. Substantial reduction in numbers of recoverable organisms was associated with drying of surfaces and satisfactory decontamination of clean worksurfaces was achieved. Both Gram-positive and Gram-negative species survived on soiled surfaces and on clean and soiled cloths for up to 4 hours and in some cases up to 24 hours. Where contaminated worksurfaces or cloths came into contact with the fingers, a steel utensil or a clean worksurface, sufficient numbers of organisms were transferred to represent a potential hazard if in contact with food.

Previous studies of contamination in domestic kitchens have indicated that contaminated wet cloths and wet cleaning utensils, together with contaminated hand and food contact surfaces, may play a significant part in the transfer of cross-contamination in the kitchen[11].

The occurrence of heavily contaminated wet cloths and the spread of contamination from these surfaces has been investigated by several workers. Scott, Bloomfield and Barlow[11] found that 48 per cent and 52 per cent respectively of domestic dishcloths and cleaning cloths showed counts greater than 100 organisms/20cm^2 ,30 per cent and 26 per cent indicating the presence of enterobacteria. De wit *et al* [3] showed that, following preparation of frozen chickens contaminated with *Escherichia coli K12* indicator organisms in kitchens, up to 74 per cent of dishcloths together with a large number of other objects in the kitchen became contaminated. They also showed that many cloths remained contaminated even after rinsing and washing.

Tebbutt[13] found that 74 per cent of cloths used for wiping surfaces in shops selling raw and cooked meats were contaminated with one or more organisms, which included *E.coli, Staphylococcus aureus, Streptococcus faecalis* and *Clostridium perfringens. E.coli* was isolated from 56 per cent of cloths, nearly half containing more than 10^5 colonies. Davies *et al* [2], Gilbert[4], Mackintosh and Hoffman[9] and Tebbutt[13] showed that wiping of hard surfaces with contaminated cloths may disseminate contamination to the hands, equipment and other surfaces.

Surface contamination in kitchens investigated by Scott *et al* [11] indicated that 10-24 per cent of domestic kitchen worktops, chopping boards, fridge and cooking hob surfaces were contaminated with greater than 200 organisms/20cm^2, 10-20 per cent showing contamination with enterobacteria. Studies in Dutch homes, where infant salmonellosis was identified, showed that kitchen sinks and work surfaces were frequently contaminated with the infecting serotype (Van Schothorst *et al*)[14]. A study by Mendes *et al* [10] of 100 catering establishments showed that 59 per cent of bacteria isolated from surfaces were of faecal origin, including *Salmonella spp.* isolated from food preparation and hand contact sites.

Despite these investigations, there has been little attempt to quantify transfer risks from contaminated surfaces. In this paper, we describe laboratory experiments which determine the extent to which survival of organisms on cloths and laminate surfaces may be associated with cross-contamination via the hands, cloths and stainless steel utensils during the course of actions mimicking those that take place in practice.

Materials and Methods

Experimental procedures are described in greater detail by Scott and Bloomfield[12]. White laminate surfaces (20cm^2), chosen to represent commonly encountered kitchen worksurfaces, were bonded with contact adhesive onto stainless steel to prevent bowing. Surfaces were swabbed with 70 per cent ethanol before use. Cloths were of the dry-woven 'J-cloth' type and were used either as 'clean' cloths (pre-wetted with saline solution to reduce absorption of contamination) or as 'soiled' cloths (previously used for washing-up in domestic kitchens for three days). Clean and soiled cloths were cut into portions (1260cm^2) and sterilised by autoclaving. A small stainless steel bowl representing a piece of kitchen equipment and fingertips were used in experiments to transfer contamination. The fingertips and bowl were swabbed with 70 per cent ethanol prior to contact with contaminated surfaces.

Experiments were performed using a range of test organisms, both laboratory strains and wild type strains of environmental origin. Gram negative and Gram positive organisms commonly occurring in kitchen environments were chosen including *Escherichia coli, Klebsiella aerogenes, Salmonella spp, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Full results for all test organisms are given elsewhere[12] and select examples are presented here. Suspensions of test organisms were prepared either in broth to simulate soiled conditions or ir

water to simulate clean conditions. Sampling of worksurfaces and cloths was performed using agar contact plates (25cm²) and the results are given in colony counts per sample area.

Survival of contamination

In experiments to determine survival of contamination on hard surfaces and on cloths, laminate surfaces and cloths were experimentally contaminated and then stored for 48 hours, by which time both the surfaces and the cloths were dry. Sampling was then begun and continued for up to a further 48 hours.

Results for experimentally contaminated worksurfaces indicate that some organisms survived for up to 24 hours on soiled surfaces. Averaged results given in Table one indicate that while both soiled and clean surfaces remained damp in the first hour following inoculation, all of the test organisms could be recovered onto contact plates to a greater or lesser extent. Some species even showed an initial increase in numbers within one hour, suggesting multiplication on surfaces. However, whereas on clean surfaces there was little survival within 4 hours, on soiled surfaces *E.coli* and *Salmonella* spp survived in significant numbers up to 4 hours and up to 24 hours for *Staph. aureus.*

Table one: Survival of contamination of worksurfaces

No. of colonies recovered per contact plate			
Escherichia coli.	**Klebsiella aerogenes**	**Salmonella spp**	**Staphylococcus aureus**
SOILED WORKSURFACE inoculated with approx 300 organisms			
Sample time			
0	139	44	190	120
1 h.	163	62	199	151
4 h.	11	3	37	98
24 h.	1	0	13	42
CLEAN WORKSURFACE inoculated with approx 300 organisms			
Sample time			
0	115	46	125	158
1 h.	109	41	131	147
4 h.	1	0	1	4
24 h.	0	0	1	2

Averaged results for experimentally contaminated cloths are given in Table two. The results indicate that for clean cloths, while numbers of organisms declined over the drying period, when sampled at four hours most organisms could still be recovered onto contact plates in substantial numbers. At 24 hours, the majority of clean cloths contained less than 20 organisms per 25cm² but for *Kl.aerogenes* regrowth occurred over 24-48 hours. Some soiled cloths showed heavy contamination at 24 hours with some indication of regrowth over 24-48 hours.

Table two: Survival of contamination of cloths

No. of colonies recovered per contact plate			
Escherichia coli.	**Klebsiella aerogenes**	**Salmonella spp**	**Staphylococcus aureus**
SOILED CLOTHS inoculated with approx 200 organisms/cm²			
Sample time			
0	T	T	T	T
1 h.	T	T	117	107
4 h.	192	201	61	12
24 h.	T	T	5	6
48 h.	T	T	0	0
CLEAN CLOTHS Inoculated with approx 200 organisms/cm²			
Sample time			
0	T	112	T	T
1 h.	T	39	T	69
4 h.	228	32	169	11
24 h.	12	5	19	1
48 h.	10	T	8	0

T: too numerous to count

Contamination transfer

In an attempt to quantify the cross-contamination risk associated with contacting either a contaminated worksurface or a contaminated cloth, experiments were devised to mimic kitchen practices which might facilitate cross contamination.

As previously, work surfaces and cloths were artificially contaminated and transfer from worksurfaces via contact with the fingertips or a small stainless steel bowl (representing a piece of kitchen equipment) was attempted at intervals up to 24 hours following contamination. Following contact with the worksurface, the contaminated fingertip or bowl was then pressed onto agar to represent transfer of contamination from one surface (i.e. worksurface) to another perhaps more critical surface such as food. Transfer from cloths was attempted via the fingertips only and the contaminated cloths were also used to wipe a clean sterile worksurface. Both the fingertips and the clean worksurface were then sampled.

Averaged results for the amount of contamination transferred from a worksurface via the fingertips or a steel bowl

are shown in Table three. The results indicate that significant numbers of all three test organisms were transferred within 1-2 hours after the worksurface had been contaminated and up to 24 hours for *Staph. aureus* via the fingertips.

The averaged results of experiments using cloths as a source of cross-contamination to fingertips and worksurfaces are given in Table four. Apart from *E.coli* transferred to fingertips and *Staph. aureus* transferred to worksurfaces at four hours, significant numbers of all three test organisms were transferred for up to 48 hours after the cloths had been contaminated.

Table three: Transfer of contamination from a soiled worksurface to fingertips or a steel bowl

DRY CONTAMINATED SURFACE approx 200-400 organisms/contact area						
	NO. OF COLONIES RECOVERED from:					
	FINGERTIP			STEEL BOWL		
	E. coli	Salmonella Spp	Staph. aureus	E.coli	Salmonella Spp	Staph. aureus
Sample time						
0	57	50	53	56	61	42
1 h.	65	66	92	52	74	43
2 h.	52	6	64	23	15	17
24 h.	3	3	19	0	0	1

Discussion

The results of this investigation illustrate the extent of bacterial survival on laminate worksurfaces and cloths. When worksurfaces and cloths were allowed to dry naturally, contamination was substantially reduced and on clean worksurfaces, drying alone produced satisfactory decontamination. However on soiled worksurfaces and on both clean and soiled cloths, organisms could be recovered for up to 4 hours after contamination occurred and in some cases up to 24 hours and 48 hours.

On cloths, there were some indications that contaminating organisms could multiply even under relatively dry conditions. These results suggest that bacterial survival and multiplication on worksurfaces and cloths depends on a number of factors (such as initial inoculum size, drying rates, presence of soil, bacterial species) and is largely unpredictable. Further experiments mimicking procedures which could lead to a cross-contamination suggest that when contaminated work surfaces come into even brief contact with the fingers or an inanimate object, significant numbers of organisms can be transferred. Similarly, where contaminated cloths were handled or applied to a clean worksurface, significant transfer is likely to occur. Transfer from cloths was greater than from worksurfaces by 24 hours and continued over a longer time period.

In assessing the hazards of cross-contamination, it must be borne in mind that the levels of contamination applied to cloths and surfaces in these investigations was relatively low compared with levels which have been reported under in-use conditions. Investigations suggest that in use contamination of cloths may range from 10^3 to 10^8 organisms/cm^2 (Davis *et al*[2], Mendes *et al*[10], Scott *et al*)[11], while Gilbert and Maurer[6] and Gilbert[5] suggest that surface contamination following contact with food may be of the order 10^2-10^6 organisms/cm^2.

The risk of infection presented by contaminated kitchen surfaces depends on the numbers of organisms transferred either directly to the mouth or more usually to a sensitive foodstuff. For salmonella, outbreaks involving chocolate and Cheddar cheese suggest that the infective dose can be as little as 50-100 and less than 10 organisms respectively (Gill *et al*[7]; Greenwood & Hooper[8]; D' Aoust)[1]. For other organisms such as toxin producers, *eg Bacillus cereus, Clostridium perfringens* and *Staph. aureus*, the accepted infective close is greater than 10^6/g food, but transfer of even small numbers of these organisms to food increases the risk of multiplication to hazardous levels under poor storage conditions.

Table four: Transfer of contamination from a cloth to fingertips and a worksurface

	NO. OF COLONIES RECOVERED from:					
	FINGERTIP			WORKSURFACE (25cm^2)		
	E. coli	K.aerogenes	Staph. aureus	E.coli	K.aerogenes	Staph. aureus
Sample time						
0	6	70	107	34	42	33
1 h.	6	47	115	26	36	28
4 h.	3	32	74	21	23	8
24 h.	T	T	T	T	T	T
48 h.	T	T	T	T	T	T

T: too numerous to count

Conclusion

From our experiments, we conclude that even relatively low levels of contamination on critical worksurfaces or cloths can present a cross-contamina-

tion hazard via contact with fingers, utensils or other clean worksurfaces. Further, while drying of surfaces and cloths can bring about a reduction in contamination levels, it cannot be relied on to prevent cross-contamination. Once again, the emphasis must be on good hand and food hygiene practices and on adequate decontamination procedures for multi-use cloths and for preparation surfaces during the handling and preparation of foods.

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FOOD POISONING
The Continuing Dilemma
20 February 1990

Dr Bernard Rowe of the PHLS and Roger Freeman, Under Secretary for Health, DoH, are among a distinguished panel of speakers addressing a seminar on food poisoning organised by the IEHO. The seminar will take place at the New Connaught Rooms, London.

Details from Susan Taylor, IEHO, Chadwick House, Rushworth Street, London SE1 0QT. Tel 01 928 6006.

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A bacteriological investigation of the effectiveness of cleaning and disinfection procedures for toilet hygiene

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The bacterial contamination of hospital and institutional toilets and toilet areas which were cleaned daily was investigated. The effect of daily disinfection with hypochlorite or a quaternary ammonium product, or with a continuous-release hypochlorite disinfectant system, based on the chlorine-releasing agent trichloroisocyanuric acid, was determined. The continuous release system produced substantial and sustained reduction in contamination of the toilet itself (water, toilet bowl and rim) and some reduction in contamination of sites surrounding the toilet (seat, floor, and air). By contrast, although daily disinfection produced some reduction in contamination compared with daily cleaning, the reductions were less than that associated with the continuous release system and indicated the inadequacy of daily disinfection and/or cleaning for toilets where effective procedures are required.

Opinions vary about the significance of bacterial contamination of toilets and other environmental sites. The assessment of potential hazard depends not only on whether the site represents a potential reservoir or an occasional source of potentially pathogenic organisms but also whether people in contact with the site are healthy adults in the community, or hospital personnel in contact with high risk patients.

Several studies have been carried out on institutional, public and domestic toilets to evaluate possible links between environmental contamination and infection. Hutchinson (1956) demonstrated the contamination of toilet seats with *Shigella sonnei* when heavily infected loose bulky stools were flushed away. In a review of cross-infection risks in hospitals, Williams *et al.* (1966) suggested that toilets could present a significant risk, although their statements were not supported by bacteriological evidence. Newsom (1972) showed that numbers of faecal bacteria recovered from well-maintained hospital toilets were low and concluded that such toilets presented little cross-infection risk. Thomas & Tillett (1973) described the role of poor conditions in junior school toilets in aiding the spread of Sonne dysentery amongst pupils. Gerba *et al.* (1975) demonstrated that flushing household toilets produces bacteria-laden aerosols which settle out on toilet and bathroom surfaces. From a study of public washrooms and toilets, Mendes & Lynch (1976) concluded that faecal bacteria occur in sufficient numbers on contact surfaces such as door, tap and flush handles to allow transfer of infection via the hands.

Whilst doubt remains about the extent of the infection risk associated with toilets, our experience suggests that many cleaning policies for hospital and public toilets still recommend some means of toilet disinfection in the belief that these procedures are associated with reduction in microbial contamination and the risk of infection.

The object of this study was to investigate the effect of cleaning on toilet hygiene in hospital and institutional toilets and compare its effectiveness with daily application of disinfectants and the use of a continuous release disinfectant system based on the chlorine-releasing reagent trichloroisocyanuric acid.

Materials and Methods

TOILETS EXAMINED

A total of twelve toilets (male and female) were monitored; six of these were at Chelsea College and six in St Stephens Hospital, London.

The College units consisted of two male and two female toilets situated in the college buildings and used throughout the day, plus two male/female shared toilets in a residential hall. These were used mostly in the morning and evening and at weekends.

The hospital units consisted of three toilets serving individual side wards (general and surgical) and a further three toilets serving a 16 bed men's ward (general, surgical and some geriatric).

SAMPLING SITES AND METHODS OF SAMPLING

Samples were taken from the water in the bowl, the bowl surface and rim, the seat and handle, the floor and the air.

Sampling procedures were based on a previously published method (Scott *et al.* 1981). Flat surfaces were sampled by placing blood agar contact plates (25 cm^2 area) in contact for 10 s. Awkward surfaces such as toilet handles and rims were sampled by nutrient agar Contact Slides (5 cm^2 area) (Tillomed Ltd, Henlow). Serum-coated swabs, pre-moistened with quarter-strength Ringer's solution, were also used to sample areas of approximately 50 cm^2 adjacent to the contact sample area. The swabs were returned immediately to their plastic containers. Toilet bowl water samples (up to 30 ml) were collected by pipette and transferred to sterile 25 ml screw-capped bottles. Water samples (10 ml) from toilets treated with chlorine disinfectants were inactivated by the addition of 0·2 ml of 0·1 molar sodium thiosulphate. Laboratory studies indicated that this concentration successfully neutralized chlorine levels of up to 5 ppm without causing inhibition of bacterial growth. Air was sampled by exposing blood agar settle plates for a period of approximately 4 h.

Samples were returned to the laboratory in an insulated cool box within 1 h of collection. Swabs were streaked onto blood and MacConkey agars. A series of dilutions of toilet bowl water samples were prepared in quarter strength Ringer's solution and 0·5 ml volumes of the appropriate dilutions were spread into blood and MacConkey agar. All plates and slides were incubated aerobically at 37°C for 24 h.

MEDIA

Except where specified otherwise, all media were prepared and supplied by Tissue Culture Services Ltd (Slough, Bucks) using media bases obtained from Oxoid Ltd.

ENUMERATION AND IDENTIFICATION OF BACTERIA

Total viable counts from bowl water samples were made by counting colonies on blood agar spread plates. Total viable counts of Gram-negative bacilli were made from MacConkey agar spread plates. Total viable counts from surface sites and air samples were made from colony counts on contact plates, slides and settle plates.

For the identification of individual species, the colonial morphology and Gram staining reactions of all isolates from blood and MacConkey contact plates and slides was noted. Gram-negative bacilli were identified by the API 20 E system for Enterobacteriacae (API) while presumptive *Staphylococcus aureus* was identified on DNase agar. *Pseudomonas aeruginosa* was confirmed with King's A media.

DISINFECTANT PRODUCTS AND PROCEDURES

The continuous-release disinfectant was a solid block trichloroisocyanurate formulation packaged to release a measured dose of approximately 4 ppm chlorine into the toilet bowl with every flush. The block is hung inside the toilet cistern and is designed to retain its effectiveness for up to 6 weeks (or 550–600 flushes). For college toilets, the daily disinfection procedure involved addition of a 10% w/v hypochlorite solution (20 ml) to the toilet. For hospital toilets, a quarternary ammonium disinfectant was applied on a daily basis. This was the product normally used in the hospital.

TEST PROGRAMME

The test programme for each toilet covered a period of 10 weeks. During the initial two-weeks period cleaning staff were requested to clean the toilets daily without applying disinfectant. In the hospital toilets a detergent was used for daily cleaning (DC), whilst in the college toilets no such products were used.

During the second two-week period, the daily disinfection (DD) procedure was carried out at both hospital and college toilets. At the end of this period the continuous release disinfectant (CRD) blocks were installed and sampling was started again after 2 d and continued for 6 weeks. During this period staff reverted to their 'normal' cleaning routine which, for hospital toilets, involved the additional use of the quaternary ammonium disinfectant product. Throughout the 10-week period, daily cleaning or disinfection, as specified, was carried out by domestic staff early in the morning before daily sampling.

For the first 7 weeks, sampling was carried out twice daily (a.m. and p.m.) on 2 d each week. For the final 3 weeks of the test period (the last 3 weeks of CRD testing), sampling was carried out once a day (p.m.) on 2 d each week.

For the first 6 weeks of the study, all sites were included in the sampling programme whilst for the final 4 weeks of the study (the last 4 weeks of CRD testing), sampling was limited to the bowl water and toilet bowl surface only.

Results

Analysis of results for male and female toilets indicated no differences and results for all toilets are therefore combined.

COLONY COUNTS

Table 1 shows the cumulative frequency of occurrence of colony counts of 48 000, 12 000, 1000, 600, 100, 10, 1 or more per ml of bowl water expressed as a percentage of samples taken during the 2 week periods of DC and DD testing and the first 2 weeks of CRD testing. Bowl water counts from hospital toilets were higher than those from college toilets and, for the latter, no attempt was made to differentiate counts greater than 1000/ml.

In college toilets, during the period of daily cleaning (DC), 27% of bowl water samples had counts of more than 600/ml. This figure was reduced to 4% in the hypochlorite DD trial and 0% in the CRD trial. Although counts of zero were recorded in 26% of the CRD treated toilets, no zero counts were recorded in the DC toilets and very few in the DD treated toilets.

In the hospital trial, 43% of samples from DC toilets had counts of 12 000/ml or more. Although this figure was reduced to 32% in the quaternary ammonium DD treated toilets, the highest counts were actually recorded from these samples (12% of counts greater than 48 000/ml). CRD-treated toilets showed a sub-

Table 1. Colony counts in toilet bowl water samples from college and hospital toilets over a 2 week period of sampling

Colony count/ml of toilet water	Cumulative frequency of occurrence as a percentage of samples taken					
	College			Hospital		
	DC	DD	CRD	DC	DD	CRD
48 000 or more	—	—	—	0	12	0
12 000 or more	—	—	—	43	32	0
1000 or more	22	4	0	79	68	2
600 or more	27	10	0	83	73	2
100 or more	49	37	2	95	83	2
10 or more	96	79	4	95	85	5
1 or more	100	94	74	95	85	19
Total number of samples	45	48	47	42	41	43

DC, daily cleaning.
DD, daily disinfection.
CRD, control release disinfectant.

Table 2. Colony counts at individual sites (other than water) for college toilets over a 2 week period of sampling

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken																	
	Bowl surface			Rim			Seat			Handle			Floor			Air		
	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD
100 or more	54	40	9	46	8	7	26	17	7	6	10	11	92	60	34	78	55	57
10 or more	93	74	55	76	56	17	85	83	72	42	82	38	100	96	100	96	100	100
1 or more	98	87	98	89	69	43	100	96	98	86	91	78	100	98	100	100	100	100
Total number of samples	46	48	47	45	38	48	46	48	47	37	30	39	40	48	47	23	22	23

DC, daily cleaning.
DD, daily disinfection.
CRD, control release disinfection.

Table 3. Colony counts at individual sites (other than water) for hospital toilets over a 2 week period of sampling

Colony counts per sample area	Cumulative frequency of occurrence as a percentage of samples taken																	
	Bowl surface			Rim			Seat			Handle			Floor			Air		
	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD
100 or more	47·5	50	8	36	44	0	22	21	10	22	29	20	88	100	83	16	0	26
10 or more	90	95	64	44	59	7	67	71	76	78	76	64	98	100	100	100	100	100
1 or more	97·5	100	96	79	100	31	95	91	98	100	93	72	98	100	100	100	100	100
Total number of samples	41	39	41	40	38	40	41	38	42	42	41	26	42	38	41	19	12	20

DC, daily cleaning.
DD, daily disinfection.
CRD, control release disinfection.

stantial reduction in colony counts with only one sample (2%) giving a count of 1000/ml or more and no growth was recorded in 81% of samples.

Tables 2 and 3 show results obtained from sites other than bowl water over the same period. For both hospital and college toilets there was a substantial reduction in the occurrence of colony counts of 100 and 10 or more per sample area for the toilet bowl, rim and seat surfaces in CRD-treated toilets when compared with DC- and DD-treated toilets. In college, but not hospital toilets, reductions were also achieved in DD toilets compared with DC toilets at these sites. Results for toilet handle, floor and air samples indicate that none of the procedures had any significant effect, although for floor and air samples there was some reductions in the occurrence of counts greater than 100.

Colony counts in CRD-treated toilet water and bowl surface samples were monitored over a total of 6 weeks, this being the recommended life of the CRD blocks. Comparisons of Table 4 with Tables 1 and 2 indicates that counts were lowest during the third and fourth weeks of monitoring but, although some increase in contamination was observed in weeks 5/6, occurrence of high counts (100 or more) remained small in comparison with toilets which were cleaned or disinfected on a daily basis. Analysis of the results from individual toilets indicated that the loss of activity for the CRD-system in the final 2 weeks was marginal and was confined to 4 heavily used toilets, the other eight blocks remaining fully effective over the 6 week period. Preliminary studies of staff facilities in a busy foodstore also indicates some loss of activity for CRD blocks in toilets where there is an excessively frequent or erratic pattern of usage although this requires further investigation.

BACTERIAL SPECIES

Although the monitoring of colony counts gives an important measure of the efficiency of cleaning and disinfection procedures, the elimination of species which may constitute an infection hazard is of equal importance.

Our investigations indicate that the range of species isolated from hospital and college toilets was similar to that found by other investigators (Newson 1972; Mendes & Lynch 1976). The most frequently isolated species were Gram-positive bacilli and micrococci but a proportion of samples both from the toilet and surrounding areas showed the presence of one or more opportunist pathogens of enteric origin including *Escherichia coli* and other enterobacteria (species of *Citrobacter*, *Enterobacter*, *Proteus* and *Klebsiella*), enterococci and *Pseudomonas aeruginosa*. The frequent isolation of *Ps. aeruginosa* from hospital but not from college toilets is in agreement with the findings of other workers (Whitby & Rampling 1972). Streptococci and other species of *Pseudomonas* were also isolated from a substantial number of college and hospital toilets, whilst *Staphylococcus aureus* was found in 2 samples taken from college toilets.

Table 5 shows the effect of cleaning and disinfection procedures on the incidence of *E. coli* and other enterobacteria, *Ps. aeruginosa* and enterococci in toilets and toilet sites. Results indicate that installation of CRD blocks pro-

Table 4. Colony counts in control release disinfectant (CRD) treated water and bowl surface samples over weeks 3–6 in college and hospital

Colony counts (per ml or per 25 cm²)	Cumulative frequency of occurrence as a percentage of samples taken							
	College				Hospital			
	Toilet water (weeks)		Bowl surface (weeks)		Toilet water (weeks)		Bowl surface (weeks)	
	3–4	5–6	3–4	5–6	3–4	5–6	3–4	5–6
100 or more	0	4	6	30	0	14	11	18
10 or more	6	21	45	70	0	23	34	55
1 or more	57	83	85	90	40	77	96	95
Total number of samples	35	24	34	23	35	22	29	22

Table 5. Frequency of occurrence of *Escherichia coli*, other enterobacteria, enterococci and *Pseudomonas aeruginosa* at toilet and surrounding sites for college and hospital toilets over a 2 week period of sampling

	Frequency of occurrence											
	College						Hospital					
	WBR			SHAF			WBR			SHAF		
Organism	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD	DC	DD	CRD
Escherichia coli	33	55	3	8	8	2	8	20	2	9	4	3
Other Enterobacteria	54	4	0	2	4	0	50	40	6	14	15	19
Enterococci	11	10	0	20	18	2	1	1	3	11	9	10
Pseudomonas aeruginosa	11	0	0	0	0	0	58	22	6	8	2	0
Total number of samples	136	134	142	146	148	156	123	118	129	144	129	129

WBR, water, bowl surface and rim sites (toilet).
SHAF, seat, handle, floor and air sites (surrounding).
DC, daily cleaning.
DD, daily disinfection.
CRD, control release disinfectant.

duced a significant reduction in the occurrence of these organisms in the toilet (bowl water, bowl surface and rim sites) compared with DC toilets. Results for DD-treated toilets also indicate some reductions in the occurrence of enterobacteria (but not of *E. coli*) and *Ps. aeruginosa* at these sites. By comparison with the toilet itself, the overall occurrence of these species at sites surrounding the toilet (seat, handle, floor and air) was relatively infrequent so that reductions in contamination were more difficult to assess and it is suggested that further samples are necessary to verify these results. Nevertheless, it was found that installation of CRD blocks produced some reduction in the occurrence of *E. coli*, enterococci and *Ps. aeruginosa* at these sites. Daily disinfection of hospital toilets with quaternary ammonium disinfectants also produced a reduction in the occurrence of *E. coli* and *Ps. aeruginosa* compared with DC toilets but disinfection of college toilets with hypochlorite had little or no effect.

Discussion

Bacteriological sampling described in this investigation indicates that even where hospital and public toilets and toilet areas are cleaned on a regular daily basis, a significant proportion of sites may be contaminated with substantial numbers of bacteria. Although the isolation of primary enteric pathogens such as shigellas and salmonellas would not be expected in a study of this size, which represents a relatively small number of samples, opportunist Gram-negative pathogens such as *Ps. aeruginosa*, *E. coli* and other enterobacteria were quite frequently isolated both from the toilet itself and from sites such as the toilet seat and handle. Overall, 54% of bowl water samples examined had bacterial counts of 600/ml or more whilst contamination levels of 100 or more organisms/25 cm^2 sample area were found on 51% of bowl surface samples. Individual counts up to $1{\cdot}2 \times 10^4$ and $4{\cdot}8 \times 10^4$/ml for *E. coli* and *Ps. aeruginosa* respectively were obtained from some toilet water samples.

Apart from contamination in the toilet itself, contamination of surrounding areas may occur due to splashing or generation of bacteria-laden aerosols during toilet flushing. Tests with toilets cleaned on a daily basis indicated that 44% of toilet seat, handle, floor and air samples had counts of 100 or more organisms/sample area, whilst results from individual toilets indicated a relationship between the occurrence of species such as *E. coli*, *Enterobacter*, *Ps. aeruginosa* in the toilet itself and their isolation from surrounding areas thereby suggesting direct transfer from the toilet.

Tests with disinfectants indicated that daily use of hypochlorite or quaternary ammonium

products produced some reduction in contamination in the toilet itself (water, bowl surface and rim), but the effects were fairly limited and generally indicated the inadequacy of daily disinfection of toilets in maintaining any real reduction in microbial contamination compared with those associated with normal cleaning.

Sampling of toilets and toilet areas following installation of CRD blocks in hospital and college toilets indicated that these systems produced substantial and sustained reductions in microbial contamination; during the first 2 week sampling period 96% of toilet bowl water samples had counts of less than 10 organisms/ml and 66% of bowl and rim samples had counts of less than 10 organisms/sample area. The CRD system appeared to be particularly effective in eliminating Gram-negative organisms from water, bowl surface and rim sites; out of a total of 266 samples, not more than 6% were found to be contaminated with a Gram-negative organism compared with 56% of DD samples. The fact that the CRD blocks produced a reduction in counts and occurrence of Gram-negative organisms on toilet seat surfaces is of interest in view of the possible role of these surfaces in the spread of infection outbreaks such as Sonne dysentery (Hutchinson 1956).

By contrast, however, the reduction in contamination at sites such as toilet handles, floor and air samples was relatively small although, for example, whereas 8 isolates of *Ps. aeruginosa* were obtained from these sites when toilets were only cleaned daily, no further isolates were obtained following installation of the CRD block. The fairly limited effects at these sites, compared with the substantial reductions in the toilet water, suggests that particularly in hospital toilet areas, a substantial proportion of the contamination arising in areas surrounding the toilet occurs by direct shedding or transfer from the patient rather than via the toilet.

Overall, our investigations indicate that although regular cleaning of hospital and institutional toilets is required to maintain them in an acceptable state, daily cleaning and/or daily disinfection produces only limited reductions in bacterial contamination of the toilet itself and of surrounding areas. On the basis of the evidence available, it is impossible, at the present time, to draw positive conclusions regarding the potential hazard associated with the presence of opportunist pathogens in toilets under normal conditions, although indications are that these are occasional and may therefore be rated as relatively low. In situations where the risk is increased (e.g. in the event of an outbreak of dysentery or salmonella infection) it may, however, be decided that decontamination of toilets is required as a means of infection control, our investigations suggest that this can be achieved only by using of an effective continuous-release or other type of sustained action disinfectant. Intermittent or occasional use of disinfectant in this situation is of little value. It must also be borne in mind that disinfection of the toilet itself produces only a limited reduction in contamination of surrounding sites, and where decontamination of toilet seats, flush handles etc is deemed necessary to prevent transfer of infection, then separate application of disinfectants is required since much of the contamination at these sites probably arises by direct shedding or contact.

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